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## CHEMICAL EXAMINATION OF THE ROOT-BARK OF ALANGIUM LAMARCKII

By

M. P. SINGH AND J. D. TEWARI

The plant *Alangium Lamarckii* belongs to the Natural Order Cornaceae and is reported to possess important medicinal properties. The part mostly used as medicine is the root-bark which is up to 2 mms. thick, brittle and dark brown in colour and highly bitter. Leaves and fruits also find use in medicine. The root-bark is described in the *Nighantu* as bitter, mucelaginous pungent, aperient; it expels worms, wind, phlegm and poison. The leaves are used externally to relieve rheumatic pains, and the fruits internally to cure phthisis and skin diseases.

From personal experience Sheriff (i) states that the bark is a good substitute for *Ipcacuanhe*, and proves useful in all cases in which the latter is indicated, except dysentery. It is given in syphilis and leprosy; the native consider it to be alexiteric abxiteric, especially in cases of bites from rabid animals. Dey. (2) reports its usefulness in simple continued fever. Chark, *Sharang-Dharsamhita* and *Bhava-prakasha* describe it as an antidote to snake venom.

The presence of a noncrystallisable alkaloid, provisionally named Alangine was reported by Dymock, (3) as far back as 1891. Later on, in 1893, a reference of the same appeared in *Chemisches Zentralblatt* (4). Chopra in 1933. (5) submitted the drug to detailed chemical study. He says, "Detailed chemical examination revealed the presence of an alkaloid and fair amount of Potassium chloride, but no tannins or glucosides. The base was purified to a great extent but all attempts to prepare a crystalline salt have thus far failed. The sulphate of the base was obtained as a white powder which was found to be hygroscopic and had a tendency to turn yellow on keeping."

Neither the alkaloid nor any one of its salts have so far been obtained in a crystalline form. It had not been possible even to decide whether the alkaloid was a single compound or a mixture of others, differing but slightly in their chemical and physical properties. The presence of characteristic groups and the nature of the nitrogen atom in the molecule of the alkaloid too, had not been studied.

In this series it is intended to throw more light on the above mentioned points. The present study was already in progress when a paper by Dutt and Parihar (6) appeared, giving results of their investigations on the drug. They report the presence of a single alkaloid—Alangine—to which they have assigned the formula  $C_{13}H_{25}O_2N$ ,—(M.P. 205–08°C. with decom.) A number of the salts of the alkaloid with their melting points are given. A few colour reactions are also given. The authors have given no reference of the previous work in the field. The colour reactions and the molecular weight that they have found out are quite different from those given by Dymock. The present authors have isolated three different alkaloids—two isomeric with each other, and a third different from these two; from the same drug, and have provisionally named them (i) Alangine A, (ii) Alangine B, and (iii) Alanginine. It is very probable, therefore, that the Alangine either of Dymock or Dutt and Parihar, is but a mixture and not a pure single individual compound. This is further supported by the fact that ethylacetate in which all the three alkaloids isolated by us, are soluble, has been used near the final stage of extraction by Dutt and Parihar. Naturally the precipitate that they obtain by adding a mixture of ether and petroleum ether (2:1) to the concentrated ethylacetate extract must also be a mixture of all three alkaloids. In the final stage of purification, the use of chloroform by them, in which solvent also all the three alkaloids are soluble, leave no room for doubt that they are dealing with a mixture and not a pure substance. The following table gives the values obtained by Dymock, present authors and Dutt and Parihar:—

Authors.	Mol. wt.	M.P.	C%	H%	N%	Dumas.	N% Kjeldahl's.	OCH <sub>3</sub> %	Mole- cular formula.
(1) Dymock	266	...	...	..	...	..	..	..	..
Dutt and Parihar.	291	205°—08°	76.25	8.36	..	4.68	4.68	10.36	C <sub>16</sub> H <sub>25</sub> —O.N.

## (3) Present Author.

i Alangine A	..	353	219°—20°	72.27	7.30	8.34	4.21	17.54	C <sub>21</sub> H <sub>25</sub> O <sub>3</sub> N <sub>2</sub>
(ii) Alangine B	...	353	105°—07°	72.20	7.41	8.18	4.15	17.49	C <sub>21</sub> H <sub>25</sub> O <sub>3</sub> N <sub>2</sub>
(iii) Alanginine	..	..	245°—47°	73.56	8.46	..	..	..	..

Furthermore the difference between the percentages of nitrogen determined by the Dumas and the Kjeldahl's methods show that in the molecule of the alkaloid nitrogen exists in two different forms and the possibility of a ring nitrogen is consequently inferred. Such a difference is not recorded by Dutt and Parihar.

## EXPERIMENTAL

Preliminary assay of the root-bark revealed the presence of about 91% of an alkaloid on the air dried material. 25 gms. of the air-dried root-bark was successively extracted with the following solvents:—

1. Petroleumether (40°—60°):—A dark brown viscous oily substance 45% was obtained.
2. Absolute ether:—A dark brown viscous oily substance 84%.
3. Absolute alcohol:—A dark brown hygroscopic mass 5.7%.
4. Alcohol 70%:—12.15%.

## Isolation:—

10 lbs. of the air dried root-bark was finely powdered and extracted with a cold homogeneous mixture of 90 parts ether, 10 parts alcohol, and 2 parts liquor ammonia, the final percolation being done after stirring up the drug with some alcoholic ammonia. The percolates were individually treated with dry hydrochloric acid gas in the cold and the ethereal solution decanted off from the precipitated hydro-

chlorides of the bases, ammoniated with liquor ammonia and used for subsequent percolation. After three percolations the ethereal solution was made ammoniacal and then just acidic with acetic acid and either distilled off on the waterbath for fresh use. From the residue left behind, the residual base was recovered by removing fatty matter with petroleum ether (40°—60°c) and dissolving out the salts with cold and finally with some warm water. The combined hydrochlorides got from the different percolates and the aqueous solutions of the salts of the residual alkaloids were mixed together, the base precipitated with sodium carbonate (1%) and shaken out with chloroform. The chloroform layer was allowed to stand for 24 hours, filtered and shaken out with 1% oxalic acid till in the last extract no precipitate was obtained by adding Meyer's reagent. From the solution of the oxalates thus obtained the bases were fractionally precipitated with ammonia shaken out with ether and three fractions collected after rationally missing the different initial fractions according to their basic solutions thus obtained were dried over anhydrous sodium sulphate strengths. The three ethereal and concentrated to a small volume over a waterbath at 50°c. A light brown solid separated in all the three and was collected over a filter paper, washed a few times with dry ether and stored as fraction A. From the respective mother liquors on largely diluting with petroleum ether a deep yellow substance was obtained and was named fraction B. The fractions A and B were further purified by repeating the above treatment and finally by dissolving them separately in dry acetone which did not dissolve a very small crop of a cream coloured substance (M. P. 245°—47° with decomposition). The acetone was removed, the solids dissolved separately in dry ether, the ethereal solution concentrated to a small volume and the base precipitated by adding large volume of petroleum ether. The three substances were obtained:—Alangin A. (M. P. 219°—20°c. with decom.), Alangine B (M. P. 105°—07°c. with decom.) and Alanginine (M. P. 245°—47°c.). They have a tendency to darken in colour in the presence of heat and sunlight. This tendency is maximum in the case of Alangine B and least in the case of Alan-

genine. The yields respectively of Alangine A, Alangine B and Alanginene on the air-dried material are  $\cdot 15\%$ ,  $\cdot 10\%$ , and  $\cdot 001\%$ .

Alangine A:—(M. P.  $219^{\circ}$ — $20^{\circ}\text{C}.$ ) is a light brown crystalline solid when freshly crystallised from hot benzene, but gradually goes on darkening in colour, becoming more and more brown under the influence of heat and light. It is soluble in chloroform, acetone, ethylacetate, ethyl and methyl alcohols, sparingly so in ether, benzene, (hot) and insoluble in cold benzene, petroleum ether and water. It yields marked precipitates with all the alkaloidal reagents and shows colour reactions as given in table 2, below. It is soluble in mineral as well as organic acid solutions, giving in all cases a light orange solution which on evaporation deposits respective salt as an amorphous jelly. The salts are extremely bitter in taste and show bluish fluorescence. From chloroform solution it deposits at once a dark brown bromine addition product when a solution of bromine in chloroform is added.

Found:—C =  $72\cdot 26$ ,  $72\cdot 28\%$ ; N =  $7\cdot 35$ ,  $7\cdot 24\%$ ; N =  $8\cdot 43$ ,  $8\cdot 25\%$  (Duma's).

N =  $4\cdot 18$ ;  $4\cdot 24\%$  (Kjeldahl's).

Mol. Weight 350; 360 (Rast's); 338·6, 338·9 (Ignition of the chloroplatinate).

— $\text{OCH}_3$  =  $17\cdot 49$ ;  $17\cdot 59\%$ . Br. in bromo compound =  $40\cdot 04$ ;  $40\cdot 23\%$ .

$\text{C}_{21}\text{H}_{25}\text{O}_3\text{N}_2$  requires:—C =  $71\cdot 38\%$ ; H =  $7\cdot 08\%$ ; N =  $7\cdot 93\%$ ;  $=\text{OCH}_3$  =  $17\cdot 56\%$  (two groups.); Br. =  $40\cdot 5\%$  (3 bromine atoms.); Mol. Weight = 353.

Alangine B:—M.P.  $105^{\circ}$ — $07^{\circ}\text{C}.$  It is a deep yellow amorphous solid when freshly obtained. It gradually changes to brick red in colour by the action of heat and light and this tendency is greater in this compound than in Alangine A. It is easily soluble in chloroform, acetone, ethyl and methyl alcohols and ethylacetate. When freshly prepared it is also easily soluble in ether and benzene but the solubility in these latter solvents goes on decreasing on keeping. Probably

that is due to a change of Alangine B to Alangine A. In other properties it is similar to Alangine A.

Found :—C=72.18; 72.05%. H=7.21; 7.13%. N=8.04, 8.12%.  
Mol. Wt.=355; 362 (Rast's). 340.6; 338.9 (ignition of Platinum Chloride.).

—OCH<sub>3</sub>=16.95; 17.13%. Br.=39.85; 40.17%.

C<sub>21</sub>H<sub>25</sub>O<sub>3</sub>N<sub>2</sub> requires:—C=71.38%, H=7.08%, N=7.93%.

—OCH<sub>3</sub>=17.56%; C<sub>19</sub>H<sub>19</sub>ON<sub>2</sub>  $\begin{cases} \text{OCH}_3 \\ \text{OCH}_3 \end{cases}$

Br.=40.5 % C<sub>21</sub>H<sub>25</sub>Br(Br<sub>2</sub>)O<sub>3</sub>N<sub>2</sub>. Mol. Wt.=353.

Alanginine:—(M.P. 245°—47°C. It is cream coloured amorphous solid. This also changes in colour on keeping but the change is very very slow. It is soluble in ethyl and methyl alcohols, but insoluble in acetone, ethylacetate, benzene and sparingly soluble in ether. The yield of the Alanginine was very small. Its properties and analysis etc., will be given later on when sufficient quantity of it, is isolated in pure form.

Further investigations, regarding structure of the compounds, are in progress.

			Table II Froehde's reagent.	Erdmanus,	Vanadium chloride.
Alangine A	...	...	Violet changing to green.	Yellowish brown	Green changing to blue.
Alangine B	..	..	Ditto	Ditto	Dirty green.
1. Indian Plants and Drugs By K. M. Nandkarni.	..	1908			P. 24.
2. Pharmacogorpha Indica By W. Dymock.	..	1891			Vol. II.
3. Indegenous group of India By Lt. Col. R. N. Chopra.	..	1933			P. 272, 273.
4. Dutt and Sinha	..			Indian Academy of Sciences.	Vol. XXIII. No. 6. 1946. P. 325.

## OXIDATION OF SULPHUR AND ITS COMPOUNDS IN LIGHT

BY N. R. DHAR AND B. V. S. RAGHAVAN

Powdered sulphur is largely used in the reclamation of alkali lands in America and Europe 20 to 30 cwts. of powdered sulphur are used per acre. In his presidential address to the National Academy of Sciences Dhar<sup>(1)</sup> stated as follows:

“In course of time the sulphur added to the soil is oxidized to sulphuric acid by the joint action of bacteria, catalysts and light and hence the sodium carbonate of alkali soil is neutralized with the formation of sodium sulphate which can be washed away by flooding the lands.” From some preliminary experiments N. R. Dhar and S. K. Mukherjee<sup>(2)</sup> concluded that when powdered sulphur is mixed with alkali soil or normal soil more sulphur is oxidized in light than in the dark under identical conditions. Purely chemical oxidation of sulphur has been reported to be a slow process<sup>(3)</sup> and it depended upon the modification of sulphur used. In this communication we are submitting the results obtained on the oxidation of finely divided sulphur, sodium thiosulphate and sodium sulphide in presence of sand both in sun light and in dark under sterile and unsterile conditions.

*Experimental* (a) 100 grs. of sand were taken in three sets in Erlenmeyer flasks to each of which 1 gr. of powdered sulphur was added. To one of the flasks  $\frac{1}{2}$  gr. of sodium bicarbonate and to a second flask  $\frac{1}{2}$  gr. of sodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 10\text{H}_2\text{O}$ ) were added and to the third flask neither carbonate nor phosphate was added. Water was added at regular intervals and the flasks were shaken. One set of flasks was kept in light and another similar set in dark by covering them with a black cloth. The amount of sulphate produced was estimated as barium sulphate making correction for the small amount of sulphate initially present in the flasks before exposure.

(b) In other experiments instead of powdered sulphur, sodium sulphide prepared by passing hydrogen sulphide into solution of

sodium hydroxide, and also sodium thiosulphate were allowed to oxidize in the presence of sand both in light and in dark and under sterile and unsterile conditions. The sterilization was carried on in an autoclave at 15 lbs. pressure for two hours. The results obtained are as follows:—

TABLE 1

Surface: 100 grs.

Exposure lasting 100 hrs.

Correction was applied for the initial amount of sulphate present.

‘ Unsterile oxidations ’

Columns

1	2	3	4	5
Materials used for oxidation.		Sulphuric acid obtained with only the oxidisable material + sand.	Sulphuric acid obtained with $\frac{1}{2}$ gr. $\text{NaHCO}_3$ + sand + oxidisable material	Sulphuric acid obtained with $\frac{1}{2}$ gr. $\text{Na}_2\text{HPO}_4$ + sand + oxidisable material
1 gr. of sulphur ...	Light	0.0221 gr	0.0241 gr	0.0251 gr
	Dark	0.0129 gr	0.0155 gr	0.0160 gr
25c.c. of hypo containing 0.5646 gr. of hypo or 0.1613 gr. of sulphur. ...	Light	0.0921 gr	0.0964 gr	0.0994 gr
	Dark	0.0678 gr	0.0686 gr	0.0697 gr
25c.c of sodium sul-phide solution containing 0.1680 gr. of sulphur. ...	Light	0.0679 gr	0.0717 gr	0.0766 gr
	Dark	0.0470 gr	0.0481 gr	0.0504 gr

TABLE 2

Surface: 100 grs.

Exposure:  $3\frac{1}{2}$  months.

(Correction was applied for the initial amount of sulphate)

(Sterile and unsterile oxidations)

1	2	3	4	5
Materials used for oxidation.		Sulphuric acid obtained with only the oxidisable matter + sand.	Sulphuric acid obtained with $\frac{1}{2}$ gr $\text{NaHCO}_3$ + sand + oxidisable material.	Sulphuric acid obtained with $\frac{1}{2}$ gr $\text{Na}_2\text{HPO}_4$ + sand + oxidisable material.
1 gr. of Sulphur	Light Unsterile	0.1662 gr	0.1997 gr	0.2094 gr
	Light Sterile	0.0881 gr	0.0968 gr	0.1193 gr
	Dark Unsterile	0.0921 gr	0.1176 gr	0.1221 gr
	Dark Sterile	0.0458 gr	0.0613 gr	0.0629 gr
	Light Unsterile	0.3977 gr	0.4115 gr	0.4496 gr
25 c.c of hypo containing 0.5654 gr. of hypo or 0.1615 gr. of sulphur.	Light Sterile	0.2236 gr	0.2447 gr	0.2522 gr
	Dark Unsterile	0.3039 gr	0.3118 gr	0.3196 gr
	Dark Sterile	0.1684 gr	0.1804 gr	0.1837 gr
	Light Unsterile	0.2859 gr	0.3033 gr	0.3124 gr
25 c.c of sodium sulphide containing. 0.1624 gr. of sulphur.	Light Sterile	0.1643 gr	0.1752 gr	0.1846 gr
	Dark Unsterile	0.1628 gr	0.1684 gr	0.1733 gr
	Dark Sterile	0.0848 gr	0.0902 gr	0.0959 gr

The experimental results show that both under sterile and unsterile conditions more sulphate is formed in the light than in the dark. Moreover in presence of sodium bicarbonate and sodium phosphate there is more of sulphate formation, than in their absence. The longer the time of exposure the greater is the amount of sulphate. These results clearly show that powdered sulphur, sodium sulphide and sodium thiosulphate can be oxidized to sulphate in presence of sand under the complete absence of bacteria; the oxidation is much accelerated by exposure to light in which the amount of oxidation is approximately double that obtained under identical conditions in the dark. Under sterile conditions, like other oxidation processes mild alkali and phosphate accelerate the oxidation of sulphur, sodium sulphide, and sodium thiosulphate both in light and in dark. It is clear therefore that when sulphur or sodium sulphide or sodium thiosulphate is added to the soil or to sand, light, micro organisms and surface facilitate their oxidation to sulphuric acid under tropical conditions.

*Summary.* These experimental results show that when powdered sulphur, sodium sulphide, sodium thiosulphate are mixed with sand they are slowly oxidized to sodium sulphate. Under sterile conditions the oxidation is less than under unsterile conditions. The oxidation is facilitated by small amounts of sodium bicarbonate and sodium phosphate. Of the three substances investigated sodium thiosulphate seems to be the most photosensitive.

**References :—**

- (1) N. R. DHAR :—Presidential address, Anniversary meeting National Academy of sciences, 1935.
- (2) N. R. DHAR & S. K. MUKHERJEE: Hill memorial thesis submitted to the Allahabad University, 1937.
- (3) WAKSMAN:—Principles of soil microbiology pages 527, 1931.

# SYNTHESIS OF HIGHER DIBASIC ACIDS FROM LOWER ONES IN THE SERIES

## PART I

Synthesis of  $\beta\beta$ -dimethyl and  $\beta\beta$ -diethyl-adipic acids

By

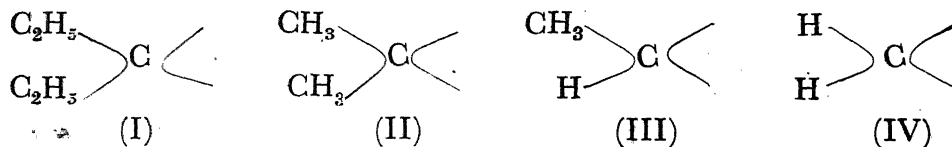
L. D. TEWARI AND J. D. TEWARI

van't Hoff in his structural theory did not postulate the carbon tetrahedron to be rigid and the directions of the valencies were what he said, only when the four groups attached to them were identical and were hydrogen atoms. (Sidgewick, 'Covalent links' page 201). A change in the directions of the valencies occurred by loading them with uneven molecules. The tetrahedral angle of Baeyer  $2 \tan^{-1} \sqrt{2} = 109.5^\circ$ , was the angle subtended at the centre by one of the sides. The change in the angle produced by substituents was calculated by Thorpe and Ingold on the basis of the molecular volume of the attached groups, Traube's<sup>1</sup> constants for the atomic volumes of methyl and carbon atoms were made use of, in determining these values.

When four hydrogen atoms were attached to the carbon atom the normal angle was  $109^\circ 28'$ . This changed with the different load

on the valencies. In  $\begin{array}{c} \text{H} \\ \theta_1 \searrow \\ \text{C} \\ \theta_2 \nearrow \\ \text{H} \end{array} \begin{array}{c} \text{C} \\ \nearrow \\ \text{C} \\ \searrow \end{array}$ ,  $\theta_2$  is greater than  $\theta_1$ , because

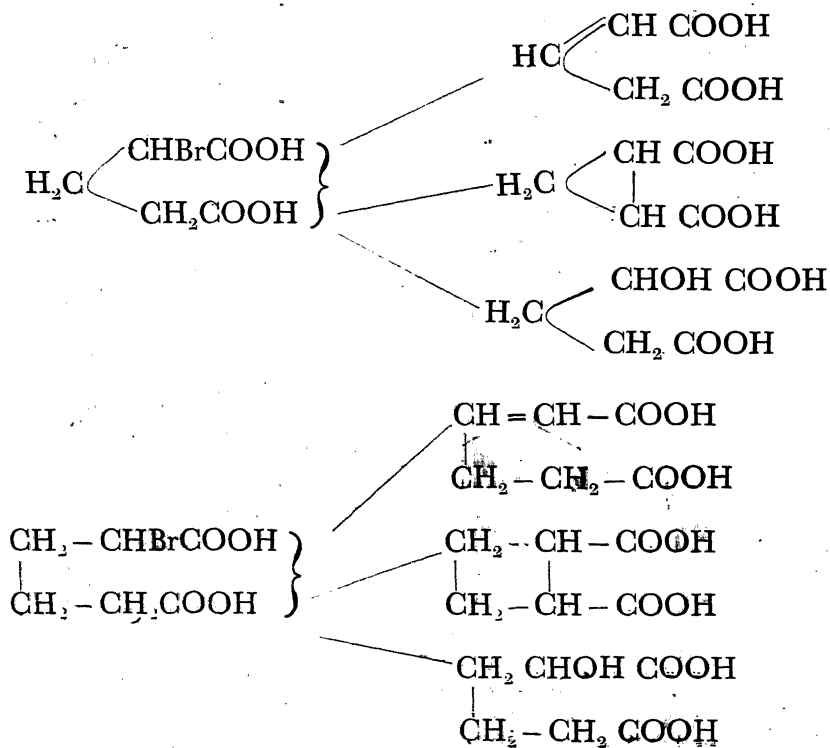
molecular volume of carbon is greater than that of hydrogen. Any attempt to reduce  $\theta_2$ , which can be done by changing  $\theta_1$ , will lead to easier formation of a carbon ring, if the arms which subtend the angle were open chains. Thorpe and Ingold have furnished experimental evidence to prove that the ease of cyclopropane ring formation was in the following order. It decreased from (i) to (iv) and was least in the case of (iv).



This is a chemical evidence on which the 'Deflexion Hypothesis' is based. The experimental value of  $\beta$  alkyl substituted glutaric acids are in agreement with the above.

$\beta$  alkyl substituted glutaric acids facilitate the formation of a three membered ring system. If in one of the arm chains attached to  $\beta$ -carbon atom in alkyl substituted glutaric acids, one more methylene group be introduced, it should facilitate the formation of a cyclobutane ring, under the conditions where  $\beta$  alkyl substituted glutaric acid yielded a cyclopropane ring.

Thus in  $\alpha$  halogenated glutaric and  $\alpha$  dipic acids the action of alkali can be postulated as follows:



Actual experiments of Ingold and other show that with dilute alkali nearly whole of the acid is converted into hydroxy acid and with concentrated alkali about 50% of cyclopropane derivative was produced contaminated with hydroxy acid and a trace of glutaconic acid, when glutaric acid was examined. In case of adipic acid, a marked difference was observed. Ethyl  $\alpha$  chloroadipate, and ethyl  $\alpha$  bromoadipate on boiling with 2N sodium carbonate yielded 90% hydroxy adipic acid. The above halogen compounds of adipic acids under exactly similar conditions as have been used in cases of similar glutaric acid compounds, on treatment with strong alcoholic (methyl) potash, gave cyclobutane dicarboxylic acid only in traces and the greater portion of the identified material was hydroxy product. The experimental evidence indicated that the formation of cyclobutane ring is attended with considerable difficulty as compared to the formation of cyclopropane ring, even when the similar methods were used for their preparation. The cyclopropane ring formation in the alkyl substituted glutaric acids was in the order predicted by Thorpe and Ingold's hypothesis.

Spiers and Thorpe<sup>2</sup> were convinced that the introduction of gem groups into malonic acid or into glutaric acid in  $\beta\beta$  position leaves the symmetry of the molecule unchanged. There is thus no free rotation effect to be considered and acids of such series are strictly comparable with one another. In succinic acid although the symmetry of the molecule is modified slightly yet the same applies to it.

It remained to be determined how similarly substituted adipic acids behaved. The field had remained unexplored for the synthesis of such adipic acids was attended with considerable difficulties.

The usual methods for the conversion of a lower dibasic acid into a higher one fail when applied for the preparations of substituted adipic acids. Then it was proposed, that attempts to introduce a methylene group in one of the free arm chains carrying carboxyl groups in glutaric acids ( $\beta\beta$ -substituted), be made. A method was devised on the lines, based on the conversion of ortho-nitro-benzoic acid into, ortho-

nitro-phenyl-acetamide<sup>3</sup>, and experiments were started to convert  $\beta\beta$ -dimethyl and  $\beta\beta$ -diethyl-glutaric acids into corresponding adipic acids.  $\beta\beta$ -dimethyl and  $\beta\beta$ -diethyl glutaric acids were prepared from acetone, diethyl ketone and cyanaceticester in ice cold alcoholic ammonia. The imide formed was hydrolysed with strong sulphuric acid, and  $\beta\beta$ -dimethyl glutaric acid was purified and converted into anhydride. The anhydride was boiled with absolute alcohol and ethylhydrogen  $\beta\beta$ -dimethyl glutarate formed was treated with thionyl chloride. The chloride formed by the replacement of the free hydroxyl group in the acid ester, was allowed to interact with diazomethane. The diazo-ketone was turned into the amide of  $\beta\beta$ -dimethyl-adipic acid by the action of ammonia and silver nitrate on its alcoholic solution. On hydrolysis the amide yielded the corresponding valerolactone instead of the desired acid and  $\beta\beta$ -diethyl glutaric acid yielded a mixture of the valerolactone and  $\beta\beta$ -diethyl adipic acid. The tendency of  $\beta$  substituted amide seems to form a six membered lactone ring in preference to acid and hence frothing during hydrolysis showing evolution of carbondioxide. From further experiments it is evident that this tendency decreases with increase in volume or complexity of the  $\beta$ -substituted group. The amide of dimethyl acid is under further investigation. The valero-lactones were converted into corresponding acids by Blanc's method<sup>4</sup>. Details of the preparations will be found in the experimental part. In Blanc's process the preparation of lactone is not easy, consequently the starting material is not easily accessible and the process becomes increasingly difficult for other  $\beta$ -substituted acids which however have been synthesised by the authors' method. In adipic acid the  $\beta$ -carbon atom from one Carboxyl group is  $\gamma$ -from another. All the alkyl substituted adipic acids synthesised are named as  $\beta\beta$ -derivatives.

Further work in connection of synthesis of other adipic acids and their bearing as a whole on the 'Valency deflection hypothesis' is in progress.

## EXPERIMENTAL

ETHYL HYDROGEN  $\beta\beta$ -DIMETHYLGLUTRATE

60 gms.  $\beta\beta$ -dimethyl glutaric acid was prepared by Thole and Thorpe's method<sup>1</sup> and was converted into its anhydride.

50 gms.  $\beta\beta$ -dimethyl glutaric anhydride was converted into ethyl hydrogen  $\beta\beta$ -dimethyl glutrate and then into its chloride by interaction with thionyl chloride<sup>2</sup>.

Two instalments of 14.4 gms. each of the chloride, were worked up for the preparation of  $\beta\beta$ -dimethyl adipic acid.

## REACTION WITH DIAZOMETHANE

14.4 gms. of chloride were dissolved in 50 c.c. pure dry ether and the solution was added to a solution of diazomethane (3 gms.) in ether (300 c.c. cooled to  $-20^{\circ}\text{C}$ ). The gradual addition of the former to the latter took about 45 minutes. The evolution of gas began to take place from the reaction mixture when the temperature rose to  $-14^{\circ}\text{C}$ . The mixture was kept over night and the next day the ether and excess of diazomethane were removed at the pump. The temperature reached by it the previous night was  $20^{\circ}\text{C}$ . The residue obtained after the removal of the solvent was treated with ammonia solution as follows:

 $\beta\beta$ -DIMETHYL ADIPIC ACID

3.4 gms. ammonia gas was dissolved in 300 c.c. alcohol at  $0^{\circ}\text{C}$  and the above residue dissolved in 50 c.c. alcohol was added to it. 7.5 c.c. 10% aqueous silver nitrate was also added and the reaction mixture was left to stand over night. Next day it was diluted with 50 c.c. alcohol and heated on a water bath. Gas evolution was noticed at  $50^{\circ}\text{C}$ . During four hours heating the temperature of the reaction mixture was raised upto  $70^{\circ}\text{C}$ . It was then filtered hot and diluted with water. The precipitated amide was digested *in situ* with 10% caustic potash and refluxed for six hours. The alkaline solution was allowed to cool. Then it was acidified with 2 normal sulphuric acid saturated with ammonium sulphate and extracted several times

with ether. The combined ethereal extract on being washed, dried with sodium sulphate and evaporated to dryness yielded a lactone, which was identified as  $\beta\beta$ -dimethyl-valero-lactone. It had a M.P.  $29^{\circ} - 30^{\circ}$  and a B.P.  $234^{\circ} - 235^{\circ}$ .

Small quantities of the lactone (2 gms. each) were sealed with potassium cyanide in glass tubes and the tubes were strongly heated. They were then allowed to cool and were cautiously opened and the contents treated with sulphuric acid. The acidic solutions were combined and saturated with ammonium sulphate. The acidic solution was extracted with ethers several times, the ethereal solution dried with sodium sulphate, and evaporated to dryness.

$\beta\beta$ -dimethyl-adipic acid thus obtained was found to contain C = 55.04 % and H = 8.0 %.  $C_8H_{14}O_4$  requires C = 55.17 %, H = 8.05%. M.P.  $87^{\circ}$ .

#### ETHYL-HYDROGEN— $\beta\beta$ -DIETHYL GLUTARATE.

60 gms.  $\beta\beta$ -diethyl glutaric acid prepared by Deshpande and Thorpe's method<sup>7</sup>, were heated under reflux with 50 c.c. acetyl chloride for three hours. Acetyl chloride was removed under reduced pressure and the residue was extracted with dry ether. The anhydride was obtained by the removal of the solvent from the ethereal extract.

50 gms.  $\beta\beta$ -diethyl glutaric anhydride was converted into ethyl hydrogen- $\beta\beta$ -diethyl glutarate, using 18 c.c. absolute alcohol and heating it under reflux. The method followed for this conversion was Qudrati-Khuda's method<sup>8</sup>.

#### CONVERSION INTO CHLORIDE

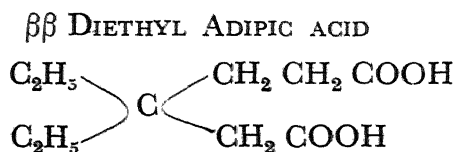
The acid ester (36 gm.) was converted into chloride by interaction with thionyl chloride (30 c.c.)<sup>9</sup>.

The chloride distilled under reduced pressure of 15—20 m.m. at  $140^{\circ} - 145^{\circ}C$ . Yield 32 gms.

It was treated with diazomethane in two successive batches 16 gms. each and then converted into amide from which the acid was obtained on hydrolysis.

## REACTION WITH DIAZOMETHANE

A solution of diazomethane (gms.) in ether (300 c.c.) was cooled to  $-20^{\circ}\text{C}$  and 16 gms. of freshly distilled chloride obtained as stated above dissolved in 50 c.c. of pure dry ether were added in drops with continual shaking to it and the addition was completed in 45 minutes. The temperature of the reaction mixture was then allowed to rise slowly. Gas evolution from it started when the temperature reached  $-12.5^{\circ}\text{C}$ . The temperature of the reaction mixture was maintained at  $-12.5^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$  till the gas evolution ceased. It took about two hours. The reaction mixture was kept over night and the maximum temperature reached by it till it was subjected to further treatment was  $20^{\circ}\text{C}$ . The solution indicated that some unreacted diazomethane was present in it and that an excess of the reagent was used. Excess of diazomethane and ether were removed at a reduced pressure and the residue obtained from two such operations was subjected to further treatment in two separate batches, till the required acid was obtained.



3.5 gms. ammonia gas was dissolved in 100 c.c. absolute alcohol at  $0^{\circ}\text{C}$ . The residue obtained from the above reaction was dissolved in 50 c.c. alcohol and mixed with ammonia solution. 7.5 c.c. of 10% silver nitrate was also added and the whole was allowed to stand over night, when it reached a maximum temperature of  $18^{\circ}\text{C}$ . It was then heated on a water bath after diluting it with 50 c.c. alcohol. The maximum temperature attained by the water bath was  $70^{\circ}\text{C}$ , as the heating was always so regulated that no vigorous evolution of gas from the solution may take place. At the end of four hours, the reaction mixture was filtered hot and diluted with water. The precipitated amide was digested with 10% caustic potash and was heated under reflux for six hours. It was allowed to cool and then acidified with 2 normal sulphuric acid. The acidified solution was saturated

with ammonium sulphate and extracted several times with ether. The ethereal extract was dried over sodium sulphate and the solvent was removed. This ethereal extract yielded a liquid residue, yellow in colour. The residue was distilled under reduced pressure and a light yellowish oil was obtained from it at  $18^{\circ}/42$  m.m. When this oil was removed from the pump and the liquid was naturally subjected to normal pressure and room temperature, white needles separated from it. It was kept in a dessicator over calcium chloride, so that the separation of the crystalline matter may reach completion. After two days the crystalline substance was separated from the oily mother liquor, washed with a little ether, dried by pressing it between filter papers, recrystallised from ether, and examined. It was found to be  $\beta\beta$ -diethyl adipic acid and melted at  $133^{\circ}-134^{\circ}\text{C}$ . It yielded a silver salt with silver content 52.0% and  $\text{C}_{10}\text{H}_{13}\text{O}_4\text{Ag}_2$  requires  $\text{Ag}=51.92\%$ . It contained  $\text{C}=59.31\%$ ,  $\text{H}=9.0\%$ ;  $\text{C}_{10}\text{H}_{13}\text{O}_4$  requires  $\text{C}=59.41\%$ ,  $\text{H}=8.91\%$ .

The liquid which remained after the removal of the acid was found to be lactone. B.P.  $136^{\circ}-138^{\circ}/20-22$  m.m.

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2. Spiers and Thorpe, J. 1925, **127**, 538.
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4. Gustave Blanc Compt. Rend, 1904, **139**, 800—802.
5. Thole and Thorpe J., 1911, 434.
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# SYNTHESIS OF HIGHER DIBASIC ACIDS FROM LOWER ONES IN THE SERIES

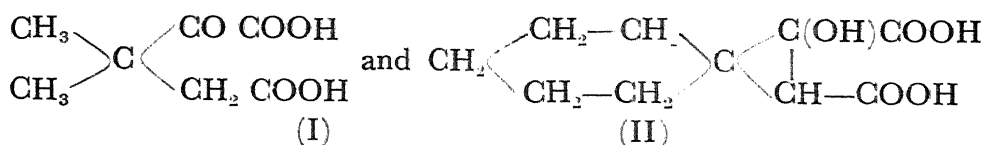
## PART II

### SYNTHESIS OF CYCLOHEXANE-I-ACETIC-I-PROPIONIC AND CYCLOPENTANE-I-ACETIC-PROPIONIC ACIDS

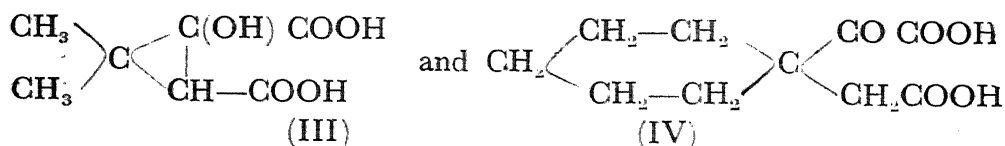
BY L. D. TEWARI AND J. D. TEWARI

Experimental data of Thorpe, Ingold and many of their co-workers showed that when the two valencies of  $\beta$ -carbon atom in glutaric acid are included in a ring complex and cyclopentane residue occupied this position, then it was found to behave as a strainless ring i.e., cyclopentane behaved just like a gem dimethyl group. Cyclohexane ring in a similar position behaved as a strained ring.

Examination of  $\beta$ -substituted glutaric acids convinced Thorpe and his co-workers that a comparatively small alteration in the angle, particularly as regards the stability of the open chain and ring system was attended with a marked difference in chemical behaviour of the compound. Thus the acids



are the stable forms though the acids



are capable of existence and it has been found possible to prepare them. It is evident that  $(\text{CH}_3)_2$  group and cyclohexane residue favour the formation of acids (I) and (II) and not of the acids (III) and (IV) respectively.

It remained to be determined how similarly substituted adipic acids behaved. Experiments were started to convert cyclohexane and cyclopentane, -I : 1-diacetic acids into cyclohexane and cyclopentane-1 acetic-i-propionic acids by the method devised in Part I of this series.

Cyclohexane-1 : 1-diacetic acid was prepared by the general method of Guareschis, by the treatment of a mixture of cyano-acetic-ester and cyclo-hexanone, with alcohol saturated with ammonia in freezing mixture at 0°. The amide formed was hydrolysed with strong sulphuric acid, and cyclohexane-1 : 1-diacetic acid obtained was purified and converted into anhydride. The anhydride was boiled with absolute alcohol, and ethyl-hydrogen-cyclohexane-1 : 1-diacetate formed was treated with thionyl chloride. The chloride formed by the replacement of the free hydroxyl group in the acid ester was allowed to interact with diazomethane. The diazo-ketone was turned into the amide of cyclohexane-1 : acetic-1 : propionic acid by the action of ammonia and silver nitrate on its alcoholic solution. The amide on hydrolysis with sulphuric acid yielded cyclohexane-1 : acetic-1 : propionic acid. Cyclopentane-1 acetic-1 propionic acid was also prepared by applying the above method using the corresponding cyclopentane compounds.

## EXPERIMENTAL

### ETHYL-HYDROGEN-CYCLOHEXANE-1 : 1-DIACETATE

100 gms. cyclohexane-1 : 1-diacetic acid prepared by Thole and Thorpe's method were heated under reflux for three hours with 80 c.c. acetyl chloride. The acetyl chloride was removed completely at the pump under reduced pressure and the remaining residue was repeatedly extracted with dry ether. The anhydride of cyclohexane-1 : 1-diacetic acid was obtained by removing the solvent from the ethereal solution.

80 gms. of cyclohexane-1 : 1-diacetic anhydride prepared as above were refluxed with 60 c.c. of absolute alcohol for five hours. A calcium chloride guard tube was attached to the open end of the reflux condenser. Excess of alcohol was removed after the refluxing was over,

at a temperature  $50^{\circ}$ - $55^{\circ}$  of water bath, under reduced pressure. The thick syrupy residue left in the distilling flask was mixed with 200 c.c. water to dilute the alcohol, if any, had remained in the ester residue. The crude ester which separated at this stage as an oily liquid was removed from the aqueous layer with the help of a separator, after taking it up in ether. The aqueous portion was extracted with ether a few times more to ensure complete removal of ester from it. The ethereal extracts were combined and then extracted several times with a solution of sodium carbonate. The combined sodium carbonate extracts on acidification with dilute hydrochloric acid liberated an oil of syrupy consistency and was ethyl-hydrogen-cyclohexane-1:1-diacetate. It was dissolved in ether and the ethereal solution was dried over sodium sulphate. The dried ethereal solution on removal of the solvent yielded pure dry ethyl-hydrogen-cyclohexane-1:1-diacetate. It showed a marked tendency to decompose on exposure. A freshly prepared sample when converted into silver salt and examined for its silver content gave a quite satisfactory value. (Obtained value  $\text{Ag} = 32.40\%$ ,  $\text{C}_{12}\text{H}_{11}\text{O}_4\text{Ag}$  requires  $\text{Ag} \equiv 32.24\%$ ).

The acid ester distilled at  $188^{\circ}$ - $190^{\circ}\text{C}$  under a diminished pressure 3-5 m.m., but the distillate was found to have undergone a considerable amount of decomposition. It appears that it is impossible to distill the acid ester even under reduced pressure, without undergoing some decomposition.

#### CONVERSION OF ACID ESTER INTO CHLORIDE

50 gms. freshly prepared ethyl-hydrogen-1:1-diacetate was mixed with 40 c.c. freshly distilled thionyl chloride slowly, with shaking, and the mixture was refluxed for three hours in a water bath, raising the temperature of water in the bath gradually upto  $80^{\circ}\text{C}$ . The reaction was allowed to complete by keeping the reaction mixture at a temperature of  $80^{\circ}\text{C}$  for about an hour. After this period, unreacted thionyl chloride was removed under reduced pressure and the residue was vacuum distilled. The chloride was obtained as a colourless mobile liquid (yield 44 gms.) B.P.  $166^{\circ}$ - $168^{\circ}$  at 4-6 m.m. pressure.

## REACTION BETWEEN THE CHLORIDE AND DIAZOMETHANE

A solution of diazomethane in 200 c.c. pure dry ether at  $-20^{\circ}\text{C}$ , was obtained from nitroso- $\beta$ -methyl amino-isobutyl methyl ketone and ten per cent caustic alcoholic potash. It represented 2.5 gm diazomethane gas and this quantity of diazomethane was decidedly more than required for the reaction.

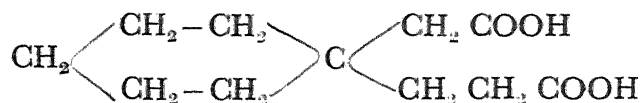
In one of the holes of a double bored thoroughly waxed cork, which exactly fitted the neck of the flask containing diazomethane solution, to form an air tight joint, a dropping funnel was inserted. Through the other bore passed a delivery tube bent twice at right angle in the same direction. The longer arm of the tube was fitted in another double bored cork, the other bore of which carried an exit tube. This cork was fitted to a second flask containing about 50 c.c. ether. A calcium chloride guard tube was fitted to the dropping funnel in the first flask, by removing its stopper. The whole arrangement was so made as to exclude any moisture from coming into contact with the reacting materials.

10 gms. freshly distilled chloride was dissolved in 50 c.c. pure dry ether and put in the dropping funnel, fitted to the flask containing diazomethane solution at  $-20^{\circ}\text{C}$  in ether. The solution of the chloride was then slowly run in, drop by drop in the diazomethane solution with continual shaking. This operation took about half an hour. The speed of addition was so regulated as not to change the temperature of the diazomethane solution appreciably. After the addition was over, the mixture was shaken thoroughly and the temperature maintained at  $-20^{\circ}$  for another half an hour. It was then allowed to rise slowly. At  $-8^{\circ}\text{C}$ , gas evolution was observed to occur in the solution. The gas evolution of the reaction mixture was allowed to complete at a temperature  $-8^{\circ}\text{C}$  to  $-5^{\circ}\text{C}$ . The reaction mixture was then left to stand over night, and the maximum temperature attained by it during that period was  $18^{\circ}$ . At the end of this time the colour of diazomethane still persisted, as decided excess of the reagent was used. The ether and excess of diazomethane were removed at the pump and the

residue was stored at a diminished pressure. 40 gms. chloride was converted into the diazo-compound in small batches of 10 gm. each and the combined residue was used for further reaction, in two instalments.

## 2

## CYCLOHEXANE-1-ACETIC-1-PROPIONIC ACID



4 gms. ammonia gas liberated from 6 N ammonia and caustic Soda, was dissolved in 120 c.c. absolute alcohol at a temperature 0°C. The residue obtained after the interaction of diazomethane and the chloride (20 gm.) was dissolved in 60 c.c. alcohol mixed with above ammonia solution. 8 c.c. of 10 % silver nitrate solution was then added and the reaction mixture was allowed to stand over night. Next day 60 c.c. ordinary alcohol was added to the solution to decrease the concentration of ammonia, and the solution was then warmed on a water bath. The heating was continued and the reaction was never allowed to become vigorous. The maximum temperature reached at the end of four hours was 70°C. Then the gas evolution from the solution ceased. The reaction mixture was filtered hot. The filtrate on dilution with water deposited the amide in a very fine form and it settled down very slowly. It was hydrolysed with 10 % caustic postash by heating it under reflux with alkali for five hours. The alkaline hydrolysis mixture was cooled and acidified with 2 normal sulphuric acid. The acidified solution was saturated with ammonium sulphate and repeatedly extracted with ether. The ethereal extracts were combined, dried with sodium sulphate and the solvent ether removed. A slightly sticky brownish solid mass was thus obtained. It was washed with small quantities of chloroform, which dissolved the coloured substance and a little of the solid as well. The chloroform extract on evaporation left a liquid residue. The white solid left after chloroform treatment was washed with petrol ether in which it was insoluble and was crystallised from benzene. It was very soluble in ether and

dissolved in hot water, and was cyclohexane-1-acetic-1-propionic acid. M.P. 169°C. It forms a silver salt which explodes on heating. (Obtained Ag=50.45 %,  $C_{11}H_{16}O_4Ag_2$  requires Ag=50.47 %. On combustion the compound gave the value, C=61.62 %, H=8.39 %:  $C_{11}H_{18}O_4$  requires C=61.68%, H=8.41 %.

## 3

## ETHYL-HYDROZEN-CYCLOPENTANE DIACETATE

60 gms. cyclopentane-1 : 1-diacetic acid prepared by Norris and 3 Thorpe's method, was converted into its anhydride by heating it under reflux for three hours with 50 c.c. acetyl chloride. After the removal of acetyl chloride the residue was extracted with dry ether and the ethereal extract on removal of the solvent yielded the anhydride of cyclopentane-1 : 1-diacetic acid.

50 gms. cyclopentane-1 : 1-diacetic anhydride was dissolved in 36 c.c. absolute alcohol and heated under reflux for six hours on a steam bath. 100 c.c. water was added to the residue and the precipitated oil was taken up in ether. The ethereal solution was extracted repeatedly with sodium carbonate. The alkaline extracts were combined and acidified with dilute hydrochloric acid, when the acid ester separated as an oil. It was treated with ether in which it dissolved and thus extracted from the acidified solution. The acidified solution was shaken with ether three times and combined ethereal extract was dried with sodium sulphate after washing it free from acid. The ether was then removed and the residue which was the acid ester of cyclopentane-1:1-acetic acid was tested for its purity by converting it into silver salt and determining the silver content (Ag=40.94 %,  $C_{11}H_{17}O_4Ag$  requires Ag=41.12 %). It distills with decomposition at 180°-182°/3-5 m.m.

## CONVERSION OF ACID ESTER INTO CHLORIDE

32 gms. ethyl-hydrogen-cyclopentane-1 : 1-diacetate was treated with 28 c.c. thionyl chloride and was refluxed for three hours, heating it on a water bath. The temperature of water in the bath was raised slowly to a maximum of 80°C towards the end. After the refluxing

was over, the excess of thionyl chloride was removed under reduced pressure and the residue vacuum distilled. The chloride distilled at 125°-128°/4-9 m.m. yield 33.0 gms. Two instalments of 16 gm. each were treated with diazomethane and subjected to further reactions.

#### REACTION WITH DIAZOMETHANE

A solution of 3 gms. diazomethane in 300 c.c. pure dry ether was cooled to -20°C and a solution of 16 gms. chloride prepared as above in 50 c.c. pure dry ether was slowly added during 45 minutes with continual shaking of the reaction mixture. The temperature was allowed to rise after the addition was over. When the temperature reached -15°C gas evolution from the reaction mixture was observed, and this was allowed to complete between -12°C and -15°C, by carefully manipulating the temperature. It was then left over night and the maximum temperature reached by the reaction mixture during that period was 18°C. The colour of diazomethane still persisted. Ether was removed at the pump and the residue converted into amide as follows:

#### CYCLOPENTANE-1-ACETIC-1-PROPIONIC ACID



3.5 gms. ammonia gas was dissolved in 100 c.c. absolute alcohol at 0°C. The residue obtained from the previous experiment was dissolved in 50 c.c. alcohol and was added to the solution of ammonia. 7.5 c.c. of 10 % silver nitrate was also added, and the whole was allowed to stand over night. Next day it was diluted with 50 c.c. alcohol and carefully heated on a water bath. Gas evolution was noticed at 50°C. The temperature of the bath was raised upto 70°C during three hours heating. This reaction mixture was filtered hot and then diluted with water, when the amide was precipitated as a fine powder. This amide was hydrolysed by refluxing it with 10 % caustic potash for six hours. The alkaline solution was cooled,

acidified with 2 normal sulphuric acid, saturated with ammonium sulphate and extracted several times with ether. The ethereal extracts were combined and the solvent was removed, after the extract had been washed and dried over sodium sulphate. The residue, which was semi-solid and stricky was washed with chloroform. After the chloroform washing, the solid which was left, was crystallised from ether, when it was obtained as a white crystalline substance melting at  $173^{\circ}\text{C}$ . This was cyclo-pentane-1-acetic, -1-propionic acid. It formed a silver salt. ( $\text{Ag}=52.0\%$ , obtained;  $\text{C}_{10}\text{H}_{14}\text{O}_4\text{-Ag}_2$  requires  $\text{Ag}=52.12\%$ ). It contained  $\text{C}=59.96\%$ ,  $\text{H}=8.1\%$  :  $\text{C}_{10}\text{H}_{16}\text{O}_4$  requires  $\text{C}=60.0\%$ ,  $\text{H}=8.0\%$ .

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*N.B.*—There is difference between the melting points, the acid prepared by Norris melts at  $142^{\circ}$  and the same acid prepared by the authors by different method melts at  $169^{\circ}$ . Impurity may be the cause, the analysis of the amide which was not separated by the authors, but hydrolysed, in situ is now under investigation and the authors are now preparing the acid by Norris method also, and a comparison of the two samples prepared by different methods will reveal their identity or otherwise.

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# COMPARATIVE EXPERIMENTS WITH INORGANIC AND ORGANIC MANURES REGARDING THE NITROGEN POSITION OF THE SOILS.

## PART I

N. R. DHAR AND B. G. CHATTERJEE

### SUMMARY

1. Cowdung (farmyard manure), neem leaf (*Melia azadirachta* Linn) and other carbonaceous substances when added to the soil fix nitrogen of the air and improve the nitrogen status, whilst with ammonium sulphate the nitrogen status remains stationary. By adding two doses of these carbonaceous substances the nitrogen content of a soil in Allahabad was raised from 0.0386% to 0.09% with wet cowdung at the rate of 68 tons per acre, with neem leaf at the rate of 30 tons per acre it went upto 0.0815 %, whilst with ammonium sulphate even at the rate of 742 lbs. of nitrogen per acre there was no increase of total nitrogen after three months. Even with 1484 lbs. per acre the nitrogen status was not improved.

2. These results obtained in India are in agreement with those obtained in the classical experiments at Rothamsted.

On the application of farmyard manure in Rothamsted fields for a number of years the soil nitrogen which was originally 0.122% rose to 0.256% from 1842 to 1914, whilst the soil without any manure fell to 0.095%. With complete artificials the status of nitrogen was 0.099%. Russell<sup>1</sup> has reported that the yield of barley and straw with farmyard manure in the Rothamsted field experiments was better than that obtained with complete artificial manures. The following data<sup>2</sup> from the Rothamsted fields present a clear picture of the nitrogen position of some of the plots:—

Total nitrogen balance sheet. (1865—1914) in top nine inches of soil,

	Farmyard manure.	No manure	Complete Plot 7	Artificial Plot 13
Total nitrogen in soil in 1865 in lbs. per acre.	4850	2960	3390	3320
Total nitrogen in soil in 1865 per cent.	0.196	0.114	0.123	0.121
Total nitrogen in soil in 1914 in lbs. per acre.	5590	2570	3210	3240
Total nitrogen in soil in 1914 in per cent.	0.236	0.092	0.120	0.122
Total change in 49 years in lbs. per acre.	+ 740	- 390	- 180	- 80

In order to find out whether these results are reproducible under Indian conditions, experiments have been taken up at Allahabad in the laboratory and under field conditions. Ammonium sulphate, freshly plucked neem leaves (*Melia azadirachta* Linn), fresh cowdung, oil cake and straw were added to the soil in different concentrations and the doses were repeated when the carbon/nitrogen ratio of the soil treated previously with manures attained the value of 10:1.

#### *Experimental.*

1. 250 grms. of soil were placed in 6 dishes and 6 pyrex glass flasks. About 40, 20, and 10 grms. of cowdung were added to various dishes and flasks and thoroughly mixed. The original C and N contents of these mixtures were estimated by the modified Kjeldahl method<sup>3</sup>. For experiments under sterile conditions the flasks were sterilised in an autoclave at 15 lbs. pressure for 30 minutes and the sets were then placed in light and in the dark by covering them with black cloth.

2. Similar experiments were carried on with different concentrations of neem leaves. The doses added were 15 grms., 8 grms., and 3.5 grms. to 250 grms. of soil.

with neem leaf was 32 millions and 122 millions, though the efficiency in both the cases was approximately double in light than in dark. The following medium was used for Azotobacter counts:—

Tap water	...	1 litre.
Mannite	...	20 grms.
K <sub>2</sub> HPO <sub>4</sub>	...	0.2 gm.
Agar	...	20 grms.

Sanai (*Crotalaria Juncea*—recommended as an excellent manure by the agricultural department)<sup>5</sup> and wheat straw were also tried in dishes mixed with soil. They are oxidised very slowly. The efficiency with wheat straw is 24 in light and 11.2 in the dark, whilst the efficiency in case of sanai is low, 5.7 in light and 3.3 in dark.

Ammonium sulphate when added to the soil gets completely lost in three months mostly as nitrogen gas during the formation and decomposition of the unstable substance ammonium nitrite<sup>6</sup>. Carbonaceous matter apart from nitrogen fixation acts as a protector of nitrogen when added with artificial manures. 10 grms. of cowdung in 150 grms. of soil reduced the loss of 0.06% of added ammonium sulphate nitrogen from 69.16% to 44.3% in one month.

*A typical table showing the fixation of nitrogen by the oxidation of cowdung.*

First application of cowdung in the field.

56Kg. of cowdung in plot 4' × 4' = 136 tons. per acre.

#### EXPOSED

Date	Total—C.%	Total—N%.	Efficiency
23-5-'47	...	1.446	0.0920
25-6-'47	...	1.304	0.0941

#### COVERED

23-5-'47	...	1.452	0.0928
25-6-'47	...	1.372	0.0934

28 Kg. of cowdung in plot of  $4' \times 4' \equiv 68$  tons. per acre.

#### EXPOSED

Date	Total—C%	Total—N%	Efficiency
24-5-'47	... 0.932	0.0652	
26-6-'47	... 0.801	0.0673	16.2

#### COVERED

Date	Total—C%	Total—N%	Efficiency
24-5-'47	... 0.946	0.0678	...
26-6-'47	... 0.871	0.0681	8.0

Second application of cowdung in the field.

56 Kg. of cowdung in  $4' \times 4'$  plot  $\equiv 136$  tons per acre.

#### EXPOSED

Date	Total —C%	Total—N%	Efficiency
15-11-'47	... 2.088	0.1458	...
10-3-'48	... 1.596	0.1517	12.0

#### COVERED

15-11-'47	... 2.146	0.1476	...
10-3-'48	... 1.865	0.1496	7.1

28 Kg. of cowdung in  $4' \times 4'$  plot  $\equiv 78$  tons per acre.

#### EXPOSED

Date	Total—C%	Total—N%	Efficiency
15-11-'47	... 1.1214	0.0856	...
10-3-'48	... 0.893	0.0901	14.1

#### COVERED

15-11-'47	... 1.249	0.0942	...
10-3-'48	... 1.064	0.0956	7.5

*A typical table showing the fixation of nitrogen by the oxidation of neem leaves*

12 Kg. of neem leaves in  $4' \times 4'$  plot  $\equiv 36$  tons per acre.

100 grms. of soil (C% - 0.386; N% - 0.0386) + 0.2828 grm. of ammonium sulphate. (0.06% nitrogen).

Condition	Hours Of exposure	Date	Total— C%	Total— N%	% Loss
Exposed ...	0	16-8-'47..	0.386	0.0986	...
Covered ...		...	0.386	0.0986	...
Exposed ...	200	18-9-'47..	0.384	0.0571	69.16
Covered ...		...	0.386	0.0709	46.16
Exposed ...	600	27-11-'47	0.384	0.0384	100.0
Covered ...		...	0.384	0.0386	100.0

100 grms. soil + 0.1414 grm. of ammonium sulphate. (0.03% nitrogen.)

Exposed ...	0	16-8-'47	0.386	0.0686	...
Covered ...			0.386	0.0686	...
Exposed ...	200	18-9-'47	0.384	0.0434	84.00
Covered ...			0.384	0.0507	59.67
Exposed ...	600	27-11-'47	0.384	0.0384	100.00
Covered ...			0.384	0.0390	98.70

The forgoing results show conclusively that repeated application of cowdung and neem leaf enrich the soil markedly by increasing the nitrogen by fixation as well as retaining the added nitrogen whilst ammonium sulphate does not improve the soil nitrogen position. These results are in agreement with those obtained in Rothamsted.

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# LOSS OF NITROGEN IN BACTERIAL NITRIFICATION OF NITROGENOUS MANURES

## PART I

N. R. DHAR AND M. C. PANT

Russell<sup>1</sup> has stated that "The essential facts of nitrification are readily demonstrated by putting a small quantity of soil—0.2 to 0.5 gm. into 50 c.c. of a dilute solution of ammonium sulphate containing nutrient inorganic salts and some calcium or magnesium carbonate but no other carbon compound. After three or four weeks at 25° the ammonia is all gone and its place is taken up by nitrates. The conversion is almost quantitative, only an insignificant quantity of nitrogen being retained by the organism."

In several publications since 1934, Dhar<sup>2-6</sup> and coworkers have recorded marked loss of nitrogen during nitrification of organic and inorganic nitrogenous manures. Similarly the experiments carried on in Rothamsted<sup>7</sup> fields show that when about 100 lbs. of nitrogen per acre as ammonium sulphate are added about 65% are lost without benefit to the soil or crop. We have explained this loss on the view point that in the process of nitrification in nature the unstable substance ammonium nitrite is formed and this decomposes<sup>8</sup> readily into nitrogen gas and water. Moreover we have emphasized that the amount of nitrate produced in a soil on the addition of a nitrogenous manure need not be the measure of the amount nitrified, as the loss of nitrogen in the gaseous state usually exceeds the amount of nitrate obtained in nitrification.

In order to test whether a similar loss of nitrogen during the bacterial nitrification of nitrogenous manures takes place we have undertaken a systematic research on this line.

## EXPERIMENTAL:—

The following culture medium was prepared for the growth of nitrifying bacteria and the nitrification to take place.

Potassium phosphate ( $K_2 HPO_4$ ).	.....1.0 gram.
Sodium chloride ( $NaCl$ )	.....2.0 grams.
Ferrous sulphate ( $FeSO_4$ )	.....0.4 gram.
Distilled water	.....1000 c.c.
Magnesium carbonate ( $MgCO_3$ ).	.....Excess. (0.5 gm. per 100 c.c.)

50 c.c. of this culture solution was placed in 300 c.c. conical flasks (Pyrex) after thoroughly shaking it. The flasks were plugged with absorbent cotton wool and were sterilized in an autoclave at 15 lbs. pressure for 30 minutes. A 10% solution of ammonium sulphate was prepared and sterilized separately. From 1 c.c. to 10 c.c. of this ammonium sulphate solution was added to various flasks with the culture solution by a sterilized pipette. 1 gram fresh garden soil was inoculated in each flask. The flasks were then incubated at  $35^\circ$  ( $\pm 0.5^\circ$ ) for a period of 7 weeks and then analysis was made by the following method.

The solution after incubation was leached with potassium chloride water (5%) and after filtration was made upto 250 c.c. The ammoniacal nitrogen left was estimated by developing colour with Nessler's reagent and comparing with a standard solution (0.0004% nitrogen) of ammonium sulphate by a Duboscq colorimeter.

50 c.c. portion of the leached solution was taken in a flask and distilled with dilute solution of caustic soda (1%). The ammonia liberated was trapped in dilute sulphuric acid and was estimated colorimetrically. Now to this distilled solution was added Devarda's alloy (2.0grams.) and 25 c.c. of 1% caustic soda solution and the flask was well corked and kept overnight. The nitric nitrogen was reduced to ammonia. This was again distilled next day and nitrogen was estimated as above. Thus the ammoniacal and nitric nitrogen were estimated. The following results were obtained:—

TABLE—I. With ammonium sulphate.

S. N.	Original Soln. in c.c.	N content N-gram	NH <sub>3</sub> -N left	NO <sub>3</sub> -N formed	Total-N left	Total-N loss	% Loss
1	10.0	0.2121	0.1328	0.0178	0.1506	0.0615	28.9
2	7.0	0.1485	0.0776	0.0160	0.0936	0.0549	37.1
3	5.0	0.1061	0.0289	0.0152	0.0441	0.0620	58.4
4	2.0	0.0424	0.0031	0.0118	0.0149	0.0275	64.6
5	1.5	0.0318	0.0019	0.0080	0.0099	0.0219	68.5
6	1.3	0.0275	0.0008	0.0041	0.0049	0.0226	81.8
7	1.0	0.0210	0.0005	0.0034	0.0039	0.0171	82.0

Similar experiments were performed with ammonium persulphate and ammonium phosphate solutions as source of ammoniacal nitrogen and the following results were obtained after analysis.

TABLE—II. With ammonium persulphate.

S. N.	Original Soln. in c.c.	N content N-gram	NH <sub>3</sub> -N left	NO <sub>3</sub> -N formed	Total-N left	Total-N loss	% Loss
1	10.0	0.3158	0.2428	0.0310	0.2738	0.0420	13.3
2	7.0	0.2211	0.1472	0.0220	0.1692	0.0519	23.1
3	5.0	0.1579	0.0888	0.0182	0.1070	0.0509	32.2
4	3.0	0.0347	0.0272	0.0142	0.0414	0.0533	56.3
5	1.0	0.0316	Trace	0.0113	0.0113	0.0203	64.2

TABLE—III. With ammonium phosphate.

S. N.	Original Soln. in c.c.	N content N-gram	NH <sub>3</sub> -N left	NO <sub>3</sub> -N formed	Total-N left	Total-N loss	% Loss
1	7.0	0.1485	0.0714	0.0180	0.0894	0.0591	39.8
2	5.0	0.1061	0.0328	0.0072	0.0400	0.0661	62.3
3	2.0	0.0424	0.0069	0.0055	0.0124	0.0300	70.7

From the above results it can be easily seen that the original nitrogen taken and the nitrogen left after the experiments is not balanced but a considerable loss is recorded. Hence Russell's statement is not confirmed and there is considerable loss of nitrogen in bacterial nitrification as in other natural conditions when nitrogenous manure is added to soil.

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## **GROWTH OF YEAST IN VARIOUS ORGANIC AND INORGANIC FOOD MATERIALS**

*By*

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Yeast is usually grown in sugars and malted barley. Recently we have developed a new technic by which yeast has been grown in solutions or suspensions of ethyl alcohol, starch, glycerol, oils gelatine, urea and even ammonium salts used as food materials. In this technic the yeast is grown in a suitable medium containing organic or inorganic food materials under sterile condition by passing a current of air which has been previously bubbled through strong sulphuric acid and distilled water.

It seems that in this process the starch or alcohol or glycerol or gelatine is partially oxidised to carbon dioxide and water and this energy of oxidation is utilised by the yeast for growth. A part of the organic compound is also utilised as food material for cell reproduction by the yeast.

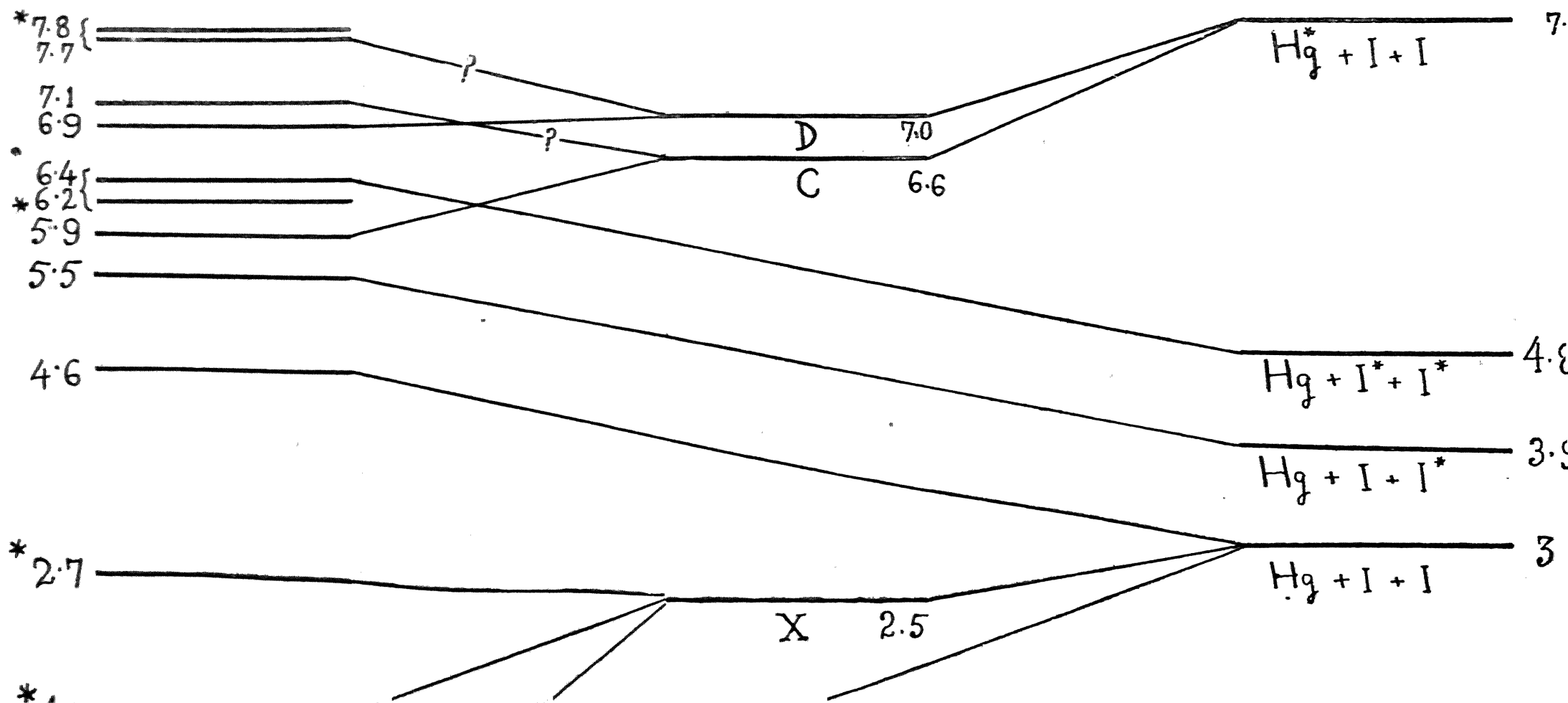
With ammonium salts by passing air in presence of yeast the energy is obtained by the oxidation of the ammonium salts to nitrite and nitrates. In this case the carbon requirement for the growth of the yeast comes from carbon dioxide of the air or from the carbonate added to the yeast. In absence of oxygen or air growth of yeast in inorganic medium seems impossible. In case the alkalinity of the medium increases on passing air yeast can not multiply and even die.

The oxidations of sodium sulphite, sodium nitrite, sodium thiosulphate, powdered sulphur, sulphuretted hydrogen solution, are being tried for the growth of yeast in the inorganic medium. It seems that this technic may be useful to bacteriologists in growing microorganisms which develop with difficulty.

# TRIATOMIC MOLECULE

# DIATOMIC MOLECULE

# ATOMIC PRODUCTS



# ENERGY LEVELS OF $\text{HgI}$ AND $\text{HgI}_2$ ITS PRODUCTS OF DISSOCIATION

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## *Abstract*

A review of the Spectroscopic work done so far in mercury iodide has been given and the energy levels of the molecules  $\text{HgI}_2$ ,  $\text{HgI}$  and their atomic products of dissociation have been plotted with respect to the ground level of  $\text{HgI}_2$  from the available thermochemical and spectroscopic data. A correlation of the levels of  $\text{HgI}_2$  with the levels of the dissociation products has been suggested in the paper.

## INTRODUCTION

For a satisfactory explanation of the spectra of triatomic molecules it is obviously an advantage to possess as complete a knowledge of the spectra of their component diatomic molecules as possible. A definite correlation between the two spectra will then be possible thus leading to a better understanding of the structure of the di- and triatomic molecules which may be derived from the related products of dissociation. The importance of such information for the theories of valency is obvious. In the case of multiatomic molecules such a procedure is much more difficult because the process of dissociation from multiatomic molecule to diatomic molecule will involve molecules of intermediate complexity about whose spectra very little is known with certainty at the present time. Yet even here a large amount of experimental work mainly on the absorption spectra of multiatomic molecules in the vapour state has been utilized for such purposes of correlation. A detailed account of such work is given in an article on "The Dissociation Spectra of Covalent Polyatomic molecules" by

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\*This is a revised form of a paper entitled "Energy levels of  $\text{HgI}_2$  and its products of dissociation" read in the Joint Session of the Indian Academy of Sciences and National Academy of Sciences at Allahabad in December 1946.

Samuel (1945). From this point of view the mercuric iodide molecule  $\text{HgI}_2$  suggests itself as a suitable molecule for investigation because both the absorption spectrum of  $\text{HgI}_2$  and the emission spectrum of  $\text{HgI}$  have received considerable attention from workers.

It is known from Raman Effect and other data that the triatomic  $\text{HgI}_2$  is linear in structure like  $\text{CO}_2$  with a frequency of valence vibration of about  $150\text{ cm}^{-1}$ \*. The absorption spectrum of the vapour of this molecule gives rise to a large number of continuous bands. The corresponding diatomic molecule  $\text{HgI}$  appears also to possess a large number of band systems which are observed only in emission and fluorescence but not in absorption.\* Many of the emission bands are not yet systematised. There is also no conclusive evidence to show whether the emitter of all these numerous emission bands is the diatomic  $\text{HgI}$  molecule or some of them are due to the triatomic  $\text{HgI}_2$  molecule.

From experiments on electronic collision with mercuric iodide vapour Pavlov and Leipunsky (1926) found four critical potentials at 1.4 volts, 2.7 volts, 6.2 volts and 7.8 volts. The first two critical potentials at 1.4 volts and 2.7 volts similar to the other two ought normally to exhibit themselves in the absorption spectrum of  $\text{HgI}_2$ . In other words the  $\text{HgI}_2$  vapour should also show two absorption bands at wavelengths 8809Å and 4568Å corresponding to these two energy values. No such absorption bands have been recorded so far

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\*\*Cf. Kohlrausch: Der Smekal-Raman-Effect; page 341 (1931 Edition)

\*It is remarkable that none of the bands observed in emission or fluorescence with mercury iodide are observed in absorption. Similar situation is met with in mercury chloride. A suggestion to explain this is made by Wieland (1945). He has calculated the equilibrium constant  $K_{-2}$  of the reactions  $\text{HgCl}_2 = \text{HgCl} + \frac{1}{2}\text{Cl}_2$ ,  $\text{HgCl} = \text{Hg} + \frac{1}{2}\text{Cl}_2$  and  $\text{HgCl}_2 = \text{Hg} + \text{Cl}_2$  from the known free energy functions of  $\text{Hg}$ ,  $\text{Cl}_2$ ,  $\text{HgCl}$  and  $\text{HgCl}_2$ . The calculations go to show that even at very high temperatures the percentage of  $\text{HgCl}_2$  molecules dissociating into  $\text{HgCl}$  and  $\text{Cl}$  is negligible and that most of  $\text{HgCl}$  molecules formed by such a dissociation are further dissociated into mercury and chlorine. It is also thought that similar results hold good for mercuric bromide and mercuric iodide.

in the absorption spectrum of mercury iodide. It may be mentioned that Kondratjew (1930, 1931) has recorded a weak absorption band in the region 4800-3900A which on experimental grounds is, however, attributed by him to an ionic mercury iodide molecule. Similarly Rao (1942) has given a very short notice of some discrete absorption bands in the region 4600-4100A which were recorded feebly in his experiments on the absorption of mercury iodide vapour and which according to him required further investigation.

An attempt in the explanation of the absorption bands has been made by Butkow (1931) and Wieland (1932) already. Both of them attribute the absorption maxima to different processes of dissociation of  $\text{HgI}_2$  into  $\text{HgI}$  and  $\text{I}$ . This is not very satisfactory. For example Wieland assumes four excited states of the  $\text{HgI}$  molecule within 4.5 e.v. of the ground level whereas in reality only two are known for certain in this range.

In view of the above facts it was thought desirable to collect the known relevant data on the spectra of the two molecules with a view to their correlation. Experiments on the absorption spectrum of  $\text{HgI}_2$  in the vapour and the solid states with a view to locate the expected band in the visible region which, however, have given a negative result are also briefly described.

#### *A Survey of Previous Spectroscopic work on mercury iodide.*

##### *(A) Work in emission and fluorescence.*

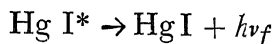
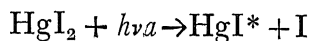
Peirce (1878) seems to have been the first person to undertake a spectroscopic study of mercuric iodide. He used Geissler discharge tubes containing the salt and on excitation found continuous bands in the region 4450—4420 A in emission. Jones (1896, 1897) extended the observations in the ultraviolet. The latter observed diffuse bands from 4396 to 2538A. The intensity of the bands had a sharp maximum at 4441A and gradually diminished towards the violet. 143 band heads were grouped in three series. Strutt (1911) found that mercury iodide when excited by active nitrogen emitted violet light which consisted of bands. Strutt and Fowler (1912) closely investigated

this spectrum and observed a weak band at 3430A which is attributed to iodine, the atomic line 2536A of mercury and a band having a very great intensity in the range 4455 to 4390A and extending up to 3700A. Chapman (1914) introduced mercuric iodide into a discharge tube of the usual type fitted with aluminium electrodes, exhausted to about .08 mm, sealed off and excited by the discharge from an induction coil. Without heating the tube the spectrum showed "the lines of mercury and iodine, besides those due to the residual air." After the tube was sufficiently heated to vaporize the mercuric iodide to a considerable extent, the character of the discharge radically changed, becoming an intense violet in colour. In addition to the lines of mercury and iodine there appeared "a brilliant band shading off towards both the red and the violet from about 4420A to 4450A."

Terenin (1926, 1927) observed a very bright visible fluorescence of mercuric iodide in the violet region when the vapour of the salt at low pressure was illuminated by a spark. The fluorescence bands had a rather sharp intensity maximum at about 4400A and a continuous falling off in intensity down to about 4000A. The lines which stimulated these bands were strictly confined to the region 2200 – 2100A. Primary dissociation of the triatomic molecule  $\text{HgI}_2$  into an unexcited binary state  $\text{HgI}$  and an iodine atom which may or may not be excited was believed to take place in the course of the excitation of these fluorescence bands.

Wieland (1929) investigated the spectrum of mercury iodide activated in Geissler tubes in the range 7000A to 2300A. He divided the bands into three systems. The first type consists of 'band heads fitting a series formula of the Deslandres type but not satisfying Mulliken's isotope equation.' These bands were ascribed to the diatomic  $\text{HgI}$  molecule. The second type of bands have a triple structure and were originally ascribed to the triatomic molecule  $\text{HgI}_2$ . In the light of the later work of Wieland (1932) in fluorescence these bands were also ascribed to the diatomic molecule  $\text{HgI}$ . Band system of the third type consists partly of diffuse bands and partly of continuous spectra.

Wieland (1932) investigated the fluorescence bands stimulated by sparks from different metals and using suitable filters. The mechanism was associated with the following equations :-



where  $h(\nu_a - \nu_f) \geq$  the dissociation energy of  $\text{HgI}_2 \rightarrow \text{HgI} + \text{I}$ . Four band systems designated by B, C, D, E in the regions 4450-3500A, 3100-2800A, 2800-2650A and 2700-~2500A respectively were observed. The following approximate formulae were suggested for the fluorescence bands :

$$\text{System C : } \nu = 32784 + (233.3\nu' - 2.25 \nu'^2) - (124\nu'' - 10\nu''^2)$$

$$\text{System D : } \nu = 36130 + 175 \nu' - 120 \nu''$$

For system B no analysis is given but the occurrence of the wave number intervals  $120 \text{ cm}^{-1}$  and  $180 \text{ cm}^{-1}$  is mentioned.

Prileshajewa (1932) observed two broad band systems in the regions 2114 to 2160A and 2168 to 2240A in an electrical excitation of mercury iodide and gave  $125 \text{ cm}^{-1}$  as the vibration frequency of  $\text{HgI}$ . Subbaraya, Rao and Rao (1937) measured the bands of system B in the first order of a 10 ft. concave grating on Rowland mounting. The spectrum was excited in emission in their experiments with a transformer discharge. The bands were found to be double-headed. The measurements of Subbaraya, Rao and Rao were found to be in close agreement with those of Terenin but were discordant with Wieland's values. The analysis of these bands as proposed by Subbaraya, Rao and Rao suggested the bands to arise due to an electronic transition between  $a^2\pi$  and  $a^2\Sigma$  state and showed that they have not got the same lower level as systems C and D. Sastry (1941, 1942) excited the mercury iodide bands in emission by both a transformer and an induction coil discharge and measured the bands of system E (in the region 2700- 2530) with a Hilger  $E_1$  quartz littrow spectrograph. He obtained in addition to the previously reported bands a new band system between 2530-2560A designated originally as system A and later by Rao, Sastry and Krishna murti (1945) as system F. The system E was

found by Sastry (1942) to consist of two components and having a common final state and an electronic width of about  $766 \text{ cm}^{-1}$ , the band heads being given by the formulae.

$$\alpha_1 \text{ System: } \nu = 38786.7 + \left[ 47.7 \left( v' + \frac{1}{2} \right) - 0.8 \left( v' + \frac{1}{2} \right)^2 \right] \\ - \left[ 55.2 \left( v'' + \frac{1}{2} \right) - 0.8 \left( v'' + \frac{1}{2} \right)^2 \right]$$

$$\beta_1 \text{ System: } \nu = 38022.1 - \left[ 44.2 \left( v' + \frac{1}{2} \right) - 0.4 \left( v' + \frac{1}{2} \right)^2 \right] \\ - \left[ 55.5 \left( v'' + \frac{1}{2} \right) - 0.8 \left( v'' + \frac{1}{2} \right)^2 \right]$$

The vibrational frequencies involved in Sastry's  $\alpha_1$  and  $\beta_1$  subsystems in the band system E are of the right order of magnitude for the deformation frequency of the triatomic  $\text{HgI}_2$  molecule. The deformation vibration of  $\text{HgI}_2$  has, however, been found to possess a frequency of  $33 \text{ cm}^{-1}$  (Sponer and Teller, 1941). It is therefore difficult to understand the above analysis of Sastry's bands.

The system F was attributed by Sastry to a transition  $^2\Pi$  to  $^2\Sigma$  showing four component heads and an electronic separation of  $126 \text{ cm}^{-1}$ . The analysis does not show any level in common with the other bands of  $\text{HgI}$ . For the  $Q_1$  heads of system F the following vibrational formula has been given:

$$\nu = 39231.1 + \left[ 98.1 \left( v' + \frac{1}{2} \right) - 2.4 \left( v' + \frac{1}{2} \right)^2 \right] \\ - \left[ 92.7 \left( v'' + \frac{1}{2} \right) - 2.0 \left( v'' + \frac{1}{2} \right)^2 \right]$$

The systems C and D were reinvestigated by Rao, Sastry and Krishnamurti (1945) who have given the following vibration formulae:

System C:

$$\nu = 32730.9 + \left[ 235.6 \left( v' + \frac{1}{2} \right) - 2.16 \left( v' + \frac{1}{2} \right)^2 \right] \\ - \left[ 125.9 \left( v'' + \frac{1}{2} \right) - 1.07 \left( v'' + \frac{1}{2} \right)^2 \right]$$

System D:

$$\begin{aligned} \nu = 36269.2 + & \left[ 178.0 \left( v' + \frac{1}{2} \right) - 1.14 \left( v' + \frac{1}{2} \right)^2 \right] \\ & - \left[ 125.70 \left( v'' + \frac{1}{2} \right) - 1.10 \left( v'' + \frac{1}{2} \right)^2 \right] \end{aligned}$$

The two systems are suggested to be the two components of a  ${}^2\Pi$  to  ${}^2\Sigma$  transition, the interval  ${}^2\Pi_{3/2} - {}^2\Pi_{1/2}$  being  $3538 \text{ cm}^{-1}$ . Rao and Rao (1946) have reported three band systems  $F_1$ ,  $F_2$ ,  $F_3$  in the regions 2550-2500A, 2435-2385A and 2345-2300A respectively.  $F_1$  comprises about 20 red degraded bands;  $F_2$  consists of a succession of closely spaced bands degraded to the red and  $F_3$  consists of about fifteen diffuse and mostly headless bands. According to them all the three systems have the same lower state, presumably  ${}^2\Sigma$ , as the systems C and D. They have also measured systems G and H (i.e. the systems originally obtained by Prileshajewa) under high dispersion and claim the existence of three distinct  $v''$  progressions in G.

Bands of systems C and D have been unanimously ascribed by all the workers to the diatomic  $\text{HgI}$  molecule. Bands of system B are double headed with a doublet separation of the order of  $60 \text{ cm}^{-1}$  and having the vibration frequency of the emitter of the order of  $270 \text{ cm}^{-1}$  and  $370 \text{ cm}^{-1}$ . No electronic level common with systems C and D has been found for system B and there is no conclusive evidence to show whether the emitter of this band system is  $\text{HgI}_2$  or  $\text{HgI}$ . Wieland interpreted these bands as having their final level the same as that of C and D systems. But Subbaraya, Rao and Rao do not agree with this view. Their data on the bands agree with those of Terenin but not with those of Wieland. The analysis of the new band systems  $F_1$ ,  $F_2$ ,  $F_3$  recently proposed by Rao and Rao obtain the band origins by extrapolation since the (0, 0) bands according to these analysis are not observed. The electronic levels of the excited states of  $\text{HgI}$  obtained from these analyses have not been taken into account in this paper for the correlation of the energy states of  $\text{HgI}_2$  and its dissociation products.

*(B) Work in Absorption.*

Chapman (1914) photographed the absorption spectrum of a solution containing mercuric iodide, potassium iodide and water. Potassium iodide was used to bring the mercuric iodide in solution. A solution of potassium iodide in water gave no absorption. Chapman claimed that there is a definite relationship between the emission and the absorption spectra, so that the vibrating system responsible for emission is responsible for absorption in solution also. Mellor\* states that Sheard and Morris (1916) found with mercuric iodide continuous regions or bands between 6072 and 5845 Å; 5605 and 5535 Å; 5195 and 5132 Å; 4828 and 4788 Å; 4506 and 4470 Å and 4450 and 4375 Å but it is not clear whether these bands were observed in emission or absorption. Most probably these observations were made in the absorption spectrum of a solution of the compound. Kondratjew (1930, 1931) observed a weak absorption in the vapour of mercuric iodide in the region 4800-3900 Å which increases in intensity with overheating of the vapour. This absorption was attributed by him to an ionic mercuric iodide molecule. The existence of this absorption is, however, doubtful as it has not been observed in any of the later investigations. Butkow (1931) investigated the absorption spectrum of mercuric iodide both as a vapour and in solution in the range 7000 Å-1950 Å. Three continuous absorption maxima at 2680 Å, 2240 Å and 2080 Å were observed. The intense maximum at 2240 Å appeared at a pressure of 0.15 mm. and the maxima at 2080 Å and 2680 Å appeared successively as the pressure was increased. Simultaneously with the appearance of the third maximum a continuous absorption with its limit at about 1950 Å appears. On a further increase of pressure the limit of the last absorption shifted towards the longer wave-lengths and finally overlapped the absorption maximum at 2080 Å. The limit of the maximum at 2680 Å was also found to shift to the longer wave-length side with increase of pressure and to reach 4400 Å at a pressure of 1-3 atmospheres. The maxima were attributed by Butkow to dif-

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\* Cf. Mellor: A Comprehensive Treatise on Inorganic and Theoretical Chemistry, Vol. 4 (1923).

ferent states of dissociation of the molecule. Thus the first and second maxima were attributed to dissociations with an unexcited  $\text{HgI}$  molecule as one product of dissociation in either case, an unexcited iodine atom being the other product of dissociation. The absorption spectra of the salt in solution clearly corresponded with that in the vapour state, the influence of the solvent being to diminish the number of maxima and to cause a slight shift of the position of the maxima. Thus the solution of  $\text{HgI}_2$  in ethanol exhibited two absorption maxima at 2725Å and 2175Å; the first obviously corresponding to the maxima at 2680Å and the second to the maxima at 2240Å and 2080Å in the vapour state. Wieland (1932) extended the observations to the far ultraviolet region upto 1500Å and found 7 to 8 absorption maxima. Continuous absorptions with the intensity maxima at 2660Å, 2240Å, 1920Å and 1720Å and banded absorptions at 2080Å and 1800Å were observed. The bands were seen to be specially sensitive to small pressure changes. Thus the absorption band at 1920Å appeared weakly at first at a pressure of 2 mms. and with increasing pressure was found to gradually spread itself and overlap the bands at 1800Å and 1720Å. The discontinuous structure of the bands at 2080Å was found to develop between the pressures 2-4 mm. From the short wavelength side commences a gradually broadening probably continuous region and above 1600Å possibly a discontinuous region whose structure overlaps the many line spectrum of hydrogen arising out of the hydrogen tube used as a source of the continuous radiation. Wehrli (1940) analysed the ultraviolet bands in the region 2066–2108Å and obtained  $156\text{cm}^{-1}$  and  $33\text{cm}^{-1}$  as the frequencies of valence and deformation vibrations in the ground state of the molecule and  $126\text{cm}^{-1}$  and  $30\text{cm}^{-1}$  the corresponding values for the excited state.

#### EXPERIMENTS ON THE ABSORPTION SPECTRUM OF $\text{HgI}_2$ IN THE VAPOUR AND THE SOLID STATES.

The usual procedure of studying the absorption spectrum was adopted in the present experiments. A sealed glass tube 9.3 cms long containing mercuric iodide in the presence of air was used for

absorption. An electric bulb or the positive crater of a carbon arc was used as the source of continuous radiations. The absorption cell could be heated to different temperatures by enclosing it in an electric furnace in the course of the experiments. The furnace was calibrated for different current strengths and the temperature of absorption cell estimated from a knowledge of the adjusted current strength and the calibration curve. Several spectrograms taken with a Hilger's Constant Deviation Glass Spectrograph failed to register any characteristic absorption by the mercuric iodide vapour. Experiments were then repeated by replacing the glass absorption cell by a quartz cell 10 cms. long. The latter had a side tube which was plugged with glass wool after being filled with mercuric iodide. No characteristic absorption of the mercuric iodide vapour in the visible region was obtained. The well known iodine absorption bands were observed to appear at about  $300^{\circ}\text{C}$  and to become more and more prominent at higher temperatures. These bands do not overlap the region of the expected band (4568A) in the visible region. The absorption bands in the near ultraviolet region, however, easily obtained on a  $\text{E}_3$  Hilger's Quartz Spectrograph when the quartz cell was used, the source of continuous radiations in this case being a locally prepared Hydrogen discharge tube with a quartz window. It has been observed that the absorption bands are practically absent if the higher temperatures are attained from room temperatures. They appear very clearly for the range of temperature  $270\text{--}300^{\circ}\text{C}$  only if the cell is first of all heated to a temperature higher than  $400^{\circ}\text{C}$  and then cooled to the required lower temperature in the range  $270\text{--}300^{\circ}\text{C}$ . No new band was observed in the quartz ultraviolet region but the plates were utilised to measure the total width of the absorption bands along with their maxima. These data are given in the following table which also records the data obtained by Wieland.

Table I

Absorption Region		Absorption Maxima	
Wavelength	e. v.	Wavelength	e. v.
2600 – 2950A	4.74 – 4.18	2700A	4.6
2330 – 2170A	5.29 – 5.68	2250A	5.5
2075 – 2090A	5.94 – 5.90	2080A	5.9
		1920A	6.4
		* } 1800A	6.9
		1720A	7.1
		[ 1600A	7.7

A further attempt to locate the expected absorption band in the visible region was made by using glass and quartz plates lightly dusted with mercuric iodide and an electric bulb or the hydrogen discharge tube as the source of continuous radiations. These experiments also yielded negative results. The near infrared region where an absorption band at 8809A is expected to lie could not be photographed as suitable plates were not available.

### Discussion

Assuming that all the observed absorption maxima belong to  $\text{HgI}_2$  and represent transitions from its ground state to various excited electronic levels, the positions of these levels are located by the energy values tabulated in column 4 of Table I.  $\text{HgI}_2$  possesses two more levels indicated by collision experiments. In the diatomic

\* Outside the range of the present experiments.

molecule  $\text{HgI}$ , so far two electronic levels in addition to the ground state are known these being at 4.1 and 4.5 e.v. relatively to the ground level. There do not appear to be any energy level lower than 4.1 e.v., the reported new bands involving levels higher than 4.5 e.v. The positions of the energy levels of  $\text{HgI}$  and of the atomic dissociation products relative to the ground level of  $\text{HgI}_2$  may be obtained if the heat of formation (or dissociation) of the two molecules in their normal states are known.

Now various methods are available to estimate the heat of formation (or dissociation) of a molecule. Thus the heat of formation of  $\text{HgI}$  molecule from its constituent atoms may be estimated from the thermochemical value of the heat of formation as is obtained from various reactions and other available data making use of the Born Cycle. It can also be obtained both in its normal and excited states making use of the analysis of the emission bands ascribed to the molecule. Here again several methods of extrapolation are available. The values obtained by all these methods for  $\text{HgI}$  in its normal and excited states are shown in Table II.

Table II\*

Level	Thermo-chemical	Linear Extrapolation	Graphical Extrapolation	Mean
X (Ground)	·58	·46 ·44	·52 ·51	·5
C (Excited)	...	·8	·85	·83
D Do	...	·86	·93	·9

\*All required thermochemical data in the present calculations are taken from Landolt-Börnstein: Physikalisch Chemische Tabellen (1935 and earlier editions) except the heat of sublimation of  $\text{HgI}$  which is taken from Sponer: Molekülspektren und ihre Anwendung Auf Chemische Probleme. The spectroscopic data are taken from Rao, Sastry and Krishnamurti. In calculating the heat of formation of  $\text{HgI}_2$ , data concerning both varieties red and yellow of  $\text{HgI}_2$  are used. The energy difference between the two varieties is about 3 KCal/Mol ( $=0.13$  e.v.) and is ignored.

Only thermo-chemical methods are available to estimate the heat of formation of  $\text{HgI}_2$  from its constituent atoms and the mean value calculated from observations of different observers taken from literature is 3.0 volts.

Making use of the absorption, collision and emission data so far known as detailed above and the calculated energies of dissociation we shall arrive at the energy level diagram shown in Fig. I for the  $\text{HgI}$  molecule and its dissociation products. The heat of formation of  $\text{HgI}_2$  from the constituent atoms in their normal states being 3 e.v. the level of the dissociated atoms  $\text{Hg} (^1\text{S})$  and  $2\text{I} (^2\text{P}_{3/2})$  is plotted 3 volts above the ground level of  $\text{HgI}_2$ . The next plausible excited states of the dissociated atoms obviously correspond to one and two excited states of the iodine atom and are plotted assuming the first excitation potential of the iodine atom to be .9 volt\*\*. Still higher excited states of the dissociated atoms correspond to  $\text{Hg}^* (^3\text{P}_1) + \text{I} + \text{I}$ ,  $\text{Hg}^* (^3\text{P}_1) + \text{I}^* (^2\text{P}_{1/2}) + \text{I}$  and  $\text{Hg}^* (^3\text{P}_1) + \text{I}^* (^2\text{P}_{1/2}) + \text{I}^* (^2\text{P}_{1/2})$  where  $\text{Hg}^*$  and  $\text{I}^*$  represent the excited states of Hg and I respectively. They are plotted assuming the first excitation potential of Hg to be 4.9 e.v.\*\*. The dissociation energy of  $\text{HgI}$  in its normal state being .5 e.v. the ground level of  $\text{HgI}$  is plotted with respect to the ground level of  $\text{HgI}_2$  by putting it .5 volt below the level of  $\text{Hg} + \text{I} + \text{I}$ . The two excited states of  $\text{HgI}$  are obtained from a knowledge of the (0, 0) bands in systems C and D viz.,  $32785 \text{ cm}^{-1}$  ( $=4.05 \text{ e.v.}$ ) and  $36295 \text{ cm}^{-1}$  ( $=4.48 \text{ e.v.}$ ).

All the three levels X, C and D of the diatomic molecule correspond to the attractive states of the molecule possessing potential energy curves with a minimum. Each of them may also represent a repulsive state for the triatomic molecule  $\text{HgI}_2$ . This seems to be much more probable for the level X which belongs to the ground state of the diatomic molecule than to the levels C and D which belong to its excited states. Among the levels of the atomic products the first one at 3 e.v. no doubt belongs to the ground states of

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\*\*Cf. Bacher and Goudsmit : Atomic Energy States.

both the diatomic and the triatomic molecules and hence definitely possesses a state of the attractive type. Similarly the one at 7.9 e.v. can be correlated within the limits of experimental and extrapolational error with the two excited states C and D of  $\text{HgI}$ . Extrapolation of C gives for the energy of dissociation .83 e.v. while that for D gives .9 e.v. (vide Table II). These give respectively 7.43 and 7.9 for the position of the level of the atomic products which are in fair agreement with the calculated value 7.9. It is, therefore, certain that the level at 7.9 e.v., also possesses a state of the attractive type. For none of the other four levels of the atomic products can this be said with certainty at the present state of our knowledge. In contradistinction to this, however, every one of these six levels will be possessing one or more states of the repulsive type. It is these repulsive states and the three states of the diatomic molecule which correlate with electronic energy states of the triatomic molecule in some way which is to be determined. To do so we come to that part of the energy diagram which belongs to the excited states of the triatomic  $\text{HgI}_2$ . We have here to bear in mind the following. The determination of the position of the excited energy levels of multiatomic molecules—probably because they possess very few stable excited states—is often not possible. In the first place most of the absorption bands are continuous. This means that the maxima represent positions on the repulsive curves belonging to the excited molecule, which are directly above equilibrium position of the normal molecule. If the repulsive curves are almost flat then the maxima would represent the positions of the levels of the dissociation products of the excited molecule, otherwise they include also an additional amount of kinetic energy, which depends on the steepness of the curves. In the few cases where band absorption is observed unless the origin of the band can be located it is not possible again to determine the position of the energy level. In this case, however, the particular potential energy curve for the molecule is an attractive one exhibiting pronounced minimum which can be located if the bands are analysed. Thus whereas band absorption has the

possibility of yielding the information about the energy levels continuous absorption gives the possibility of determining the energy of the dissociation products of the excited states. Collision data also represent a mixture of both types of excited states, repulsive as well as attractive, of the molecules. This is true as much for diatomic molecules as for multiatomic ones. Only in the case of atoms, do the collisions and absorption data give just the energy levels of the excited atoms.

Among the energy values of the triatomic molecule determined by critical potentials and absorption maxima those of 6.2 (coll.) and 6.4 (abs.) seem to be identical as also those of 7.8 (coll.) and 7.7 (abs.). These are, therefore, bracketed in the figure. We thus have nine energy values of the excited  $\text{HgI}_2$  molecule. Wieland has observed structure in the absorption maxima at 5.9 and 6.9 e.v. Under the dispersion used in our experiments we could not detect any structure in the band at 5.9 e.v. but it is particularly a very sharp and narrow band. Wehrli (1940) has analysed this band system and obtained the vibration frequencies of the triatomic molecule both in the ground state and excited state corresponding to this absorption. The region of absorption observed by him is wider than that observed in the present experiments. The conditions of his experiments could not be ascertained as his paper was not available. These two values therefore very probably represent stable excited energy states of the triatomic molecule having attractive potential curves whose dissociation limits lead to C and D respectively of the diatomic molecule  $\text{HgI}$  or to the level at 7.9 of the separated atoms. Similarly the value of 1.4 probably represents an excited energy state of the molecule because there is no energy level to which it can be correlated either in the diatomic molecule or in the separated atoms with a value less than 1.4 e.v. If the collision data are correct in establishing this level, it can be predicted with considerable confidence that there will be a banded absorption for  $\text{HgI}_2$  in the near infra-red region†

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† However dissociation of the  $\text{HgI}_2$  into  $\text{I}_2$  and  $\text{Hg}$  may also be the process brought about by collision. This process requires just 1.42 e.v.

The remaining six values almost certainly represent only positions directly above the equilibrium position in the ground state on the repulsive potential curves belonging to excited  $\text{HgI}_2$ . Of these the one at 2.7 e.v. can go over only to the level X of the diatomic molecule at 2.5 e.v.

Among the remaining five levels, the three levels at 4.6, 5.5 and 6.4 e.v. are separated by the doublet separation of the Iodine atom viz. 0.9 e.v. and hence can be correlated to the levels at 3, 3.9 and 4.8 e.v. of the atomic products with considerable confidence. From a glance at the energy level diagram it can be seen that such a correlation suggests the absorption bands corresponding to these energy levels to have widths of roughly the same order. The widths of the bands at 4.6 and 5.5 e. v. have been found to be .56 and .39 e.v. respectively in the present experiments whereas the width of the band at 6.4 e.v. (which is outside the range of the present experiments) is not recorded.

Wieland observed some structure in the absorption band at 7.7 e.v. but this has not been definitely established due to the overlapping of the many line spectrum of hydrogen with this absorption region. If the observation is correct there should be a stable level of the triatomic molecule at 7.8 e.v. going over to some higher state of the dissociation products. The latter state may be any one of the states 8.8 and 9.7 e.v. of the atomic products ; correlation with 7.9 e.v. being not very probable due to its small difference in energy with the triatomic level considered. No definite correlation for this stable state – if it exists at all – can be given without further work on the corresponding absorption band. The explanation of the excitation of the fluorescence bands of system D would necessitate the existence of a repulsive state of the triatomic molecule at 7.7 e.v. going over to the state D of the diatomic molecule. This correlation should, however, be considered as tentative ; the level D of the diatomic molecule being an excited state.

Now the level at 7.1 e.v. remains to be explained. The possibility of an intermediate level between X and C in the diatomic molecule is not very sure. The analysis of the bands of system B by various workers locate one or more levels between X and C. Different workers do not agree with one another in their analysis and measurements of this band system. Moreover there is no conclusive evidence for the identification of the emitter of this band system with the diatomic HgI molecule. Further experimental work is needed to unequivocally decide about this point. The absorption band at 7.1 e.v. being a continuous one there is a repulsive state associated with it. The only known level to which it can go is the level C of the diatomic molecule. This correlation should also be regarded as tentative. The discrepancy in the minimum values for the frequencies of the radiations exciting the fluorescence bands of systems C and D as found from this correlation with those experimentally observed by Wieland may be ascribed to the fact that the accuracy of the observations is not great, the wavelengths of the exciting radiations having been separated out with the help of suitable filters.

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# INFLUENCE OF TEMPERATURE ON NITROGEN FIXATION IN STERILE AND UNSTERILE CONDITIONS

## PART I

By

N. R. DHAR AND R. C. KAPOOR

While it has long been believed that in the protein bodies of certain organisms existing in the soil, nitrogen of the air becomes incorporated leading to enrichment of soil nitrogen<sup>1</sup>, Dhar and co-workers have produced strong evidence in favour of a purely physico-chemical view of nitrogen fixation in soil<sup>2</sup>.

Not much work have been done regarding the influence of temperature on nitrogen fixation. We have under taken a systematic investigation on nitrogen fixation at different temperatures on the addition of energy materials under sterile and unsterile conditions.

*Experimental details.* The soil or purified aluminium oxide or zinc oxide mixed with the energy rich substance was kept in 600 cc. pyrex flasks, plugged with absorbent cotton wool and kept under 16 lbs. pressure for one hour for sterilization. After every month the flasks were opened, moisture contents made up to 10% and resterilised. In each case the soil was tested for Azotobacter by the plate method and no colony growth was observed even after seven days incubation at 35°.

In these experiments 100 gms of well seaved spoil was taken in each case and 2 gms of cane sugar was mixed with it as the energy material. Moisture content was made to 10% in every case. These sterilized flasks were incubated at different temperatures in the dark. One such flask was exposed to daylight for seven hours daily for comparison. The temperature of the incubated flasks varied from 7° to 60°. Some experiments for comparison were also carried out in unsterile condition.

The carbon and nitrogen estimations were done by the method of Robinson, McLean and Williams<sup>3</sup> by heating 5 gms. of well dried and powdered soil with 30 ccs. of pure concentrated Sulphuric acid, 5 gms. of fused potassium sulphate and a few crystals of pure copper sulphate for four hours. The ammonium sulphate thus formed was estimated by treating an adequate part of it with magnesium oxide and 100 ccs water and distilled for six hours in a water bath. At the same time a current of air purified by passing through a solution of ferrous sulphate and sulphuric acid was aspirated. The ammonia was absorbed in two conical flasks containing pure dilute sulphuric acid. The ammonia obtained was estimated colorimetrically by nesslerization in a Duboscq colorimeter.

The total carbon was determined simultaneously by absorbing the sulphur dioxide produced at the time of charring by a standard iodine solution, the excess of which was titrated against a standard thio-sulphate solution.

The results with cane sugar are as follows:

I—100 gms soil + 2 gms cane sugar (Sterile)

Carbon introduced—0.8 % approximately

Time of exposure:—342 days (5-3-47 to 10-2-48)

Method of Exposure	5-3-47		22-5-47		8-11-47		10-2-48		Carbon oxidised %	Nitrogen fixed per gm. of carbon oxidised (mgm.)
	Total C %	Total N %	Total C %	Total N %	Total C %	Total N %	Total C %	Total N %		
Exposed to Light ...	1.220	0.0385	0.938	0.0429	0.658	0.0468	0.594	0.0476	0.626	14.5
7° (Dark) ...	1.220	0.0385	1.086	0.0386	0.860	0.0385	0.788	0.0385	0.432	Nil
30 (Dark) ...	1.220	0.0385	1.012	0.0404	0.728	0.0421	0.668	0.0424	0.552	7.0
35° (Dark) ...	1.220	0.0385	1.007	0.0404	0.709	0.0426	0.646	0.0430	0.574	8.6
40° (Dark) ...	1.220	0.0385	1.035	0.0393	0.792	0.0402	...	...	...	3.9
45° (Dark) ...	1.220	0.0385	1.080	0.0388	0.813	0.0393	0.754	0.0394	0.466	1.9
60° (Dark) ...	1.220	0.0385	1.095	0.0384	0.838	0.0384	0.779	0.0384	0.441	Nil

Exactly similar experiments were carried out with cowdung as energy material. However, one set in unsterile condition was also kept and the results are as follows :

II—100 gms. Soil + 10 gms. Cowdung (Unsterile)

Carbon introduced = .8% Approximately

Time of Exposure 24-12-46 to 19-1-48

Method of Exposure	24-12-46		5-4-47		26-8-47		19-1-48		Nitrogen fixed per gm. of carbon oxidised
	Total C %	Total N %	Total C %	Total N %	Total C %	Total N %	Total C %	Total N %	
Exposed to light	1.165	0.0742	0.876	0.0808	<b>0.816</b>	<b>0.0810</b>	0.780	0.0792	19.5 mg.
7° (Dark)	1.158	0.0740	1.060	0.0740	1.024	0.0740	0.968	0.0742	nil
25° (Dark)	1.162	0.0742	0.923	0.0764	0.835	0.0770	0.804	0.0772	8.4 mg.
35° (Dark)	1.160	0.0742	0.898	0.0772	0.822	0.0779	0.781	0.0780	10.0 mg.
40° (Dark)	1.162	0.0740	0.928	0.0752	0.857	0.0754	...	...	4.0 mg.
60° (Dark)	1.161	0.0741	1.116	0.0740	1.084	0.0740	0.988	0.0740	nil

*Discussion*:—From the foregoing results it is clear that the optimum temperature for carbon oxidation and nitrogen fixation is 35° in dark. The fall in carbon oxidation and nitrogen fixation after 35° is more marked than before 35°. There seems to be no nitrogen fixation at 7° and 60° though carbon oxidation takes place to some extent. It is interesting to note that similar observations of carbon oxidation in solutions have been obtained by Dhar<sup>4</sup> while studying. "The influence of temperature on the slow and induced oxidation of glucose in the dark." It is clear from the observations tabled there that "the velocity of the oxidation of glucose goes on increasing upto a certain optimum temperature, which lies between 30° and 40°.

The conclusions formulated above are made clear by the following table.

I. Soil + Cane sugar.

Carbon introduced = 0.8 % approximately

Time of exposure = 342 days

Temp.	Carbon oxidised	Efficiency of nitrogen fixation
(i) Light →	0.626% →	14.5 m/gms.
(ii) 7° dark →	0.432% →	nil "
(iii) 30° " →	0.552% →	7.0 "
(iv) 35° " →	0.574% →	8.6 "
(v) 45° " →	0.466% →	1.9 "
(vi) 60° " →	0.441% →	nil "

II. Soil + Cowdung (Sterile)

Carbon introduced = .8% approx.

Time of exposure = 245 days.

(i) Light →	.251% →	17.5 m/gms.
(ii) 7° Dark →	.130% →	nil "
(iii) 25° " →	.186% →	9.1 "
(iv) 35° " →	.221% →	9.9 "
(v) 40° " →	.164% →	5.3 "
(vi) 60° " →	.073% →	nil "

## III. Soil + Cowdung (Unsterile)

Carbon introduced = .8% approx.

Time of exposure = 245 days

(i) Light	—→	.349%	—→	19.5 m. gms.
(ii) 7° Dark	—→	.134%	—→	nil „
(iii) 25° „	—→	.327%	—→	8.6 „
(iv) 35° „	—→	.338%	—→	10.9 „
(v) 40° „	—→	.305%	—→	4.6 „
(vii) 60° „	—→	.077%	—→	nil „

Dhar and Seshacharyulu also carried on some experiments on nitrogen fixation at different temperatures in unsterile condition. In their experiments glucose was used as the energy material. The results of carbon oxidation and the efficiency of nitrogen fixation in equal time at different temperatures are given below.

Temperature	Time of exposure 27-2-37 to 13-5-37		Efficiency of Nitrogen fix. 13.1 m/gms.
	Carbon oxidised		
(i) Light	.670%		
(ii) 10°-12°	.369%		nil    "
(iii) 25°	.385%		5.4    "
(iv) 30°	.488%		6.7    "
(v) 35°	.555%		8.1    "
(vi) 40°	.374%		4.8    "
(vii) 45°	.327%		4.2    "
(viii) 50°	.254%		1.9    "
(ix) 60°	.237%		nil    "

It is significant to note that the results obtained by using glucose in unsterile conditions are similar to those obtained by us in sterile conditions by using different energy materials. Here also the carbon oxidation and the efficiency of nitrogen fixation is greater in light than

at any temperature in dark which goes to prove that it is light and not the higher temperature, that increases the fixation in exposed soil sets.

There was no fixation at 7° even in the unsterile sets though some *Azotobacter* growth was there which shows that the activity of *Azotobacter* ceases at that temperature which is in accordance with previous experiments.

In our experiments it is noted that the velocity of carbon oxidation and the efficiency of nitrogen fixation falls with time. Though the velocity of carbon oxidation varies with the nature of energy material, the amount of nitrogen fixed per gram of carbon oxidised remains nearly the same for all the energy materials at a constant temperature. This goes to prove that the energy required for nitrogen fixation comes from the energy liberated by the oxidation of carbon.

These results are best explained in the light of adsorption of gases on Solid Surfaces. The normal adsorption due to vander Waal's forces decreases with rise of temperature while the activated adsorption, since it is chemical in nature, increases with temperature just like the velocity coefficient of ordinary chemical reactions requiring a high activation energy. This explains as to why we do not get any appreciable fixation at 7°. As the temperature is increased a condition is obtained in which the rate of the activated process becomes appreciable and so the resultant total adsorption begins to increase with rise of temperature as takes place below 35. This increase continues over a range of temperature, until the normal effect of temperature on the amount adsorbed, apart from its rate, brings about a decrease, as takes place above 35°.

In our experiments the actual temperature when the rate of activated adsorption becomes appreciable lies somewhere between 7° and 25°. The activated process reaction stops at a temperature little above 45°. Further experiments on efficiency determination at the in between temperatures are in progress.

The energy of activation of the reaction  
 free nitrogen  $\longrightarrow$  fixed nitrogen  
 has been calculated as follows

$$\frac{d \log_e k}{dT} = \frac{E}{RT^2}$$

Integrating at two different temperatures

$$\log_e \frac{k_2}{k_1} = \frac{E}{R} \left\{ \frac{1}{T_1} - \frac{1}{T_2} \right\}$$

where  $k_1$  and  $k_2$  are the velocity constant of the reaction at temperature  $T_1$  and  $T_2$  respectively

$$\text{Hence } E = \frac{2.303 \log_{10} \frac{k_2}{k_1} \times R \times T_1 \times T_2}{T_2 - T_1}$$

As the velocity constants are proportional to the products formed, so here taking the cane of cane sugar at 30° and 35°

$$E = \frac{2.303 \log_{10} \frac{45}{39} \times 1.99 \times 308 \times 303}{308 - 303}$$

Since 45 and 39 are the amounts of nitrogen fixed at 35° and 30° respectively.

$$E = 5300 \text{ calories}$$

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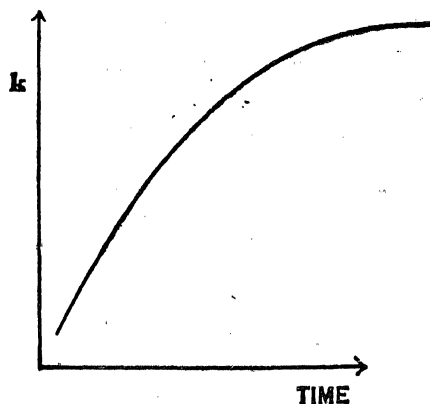
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## Calculation of Velocity Constant in Presence of Heterogeneous Auto-catalyst.

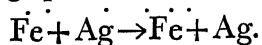
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In two previous communications (2,3) the present authors have studied the kinetics of reduction of silver acetate and silver tartrate by resorcin wherein it has been shown that the values of the velocity constant increase with time, reach a maximum and then begin to decrease. This can be well represented by the following curve:—



The ascending portion of the curve points to the autocatalytic nature of the reaction—the fine particles of silver produced acting as heterogeneous catalyst. The descending portion of the curve points to the decrease in catalytic activity due to decrease of catalytic surface caused by gradual coagulation of fine particles of silver. It requires a high mathematical ingenuity to devise a formula which shall give constant values for the velocity constant since the catalysis depends upon total surface which is a complicated function of the number and size of the particles of silver both of which are in a state of constant flux. We have failed to devise any such formula but have worked out an equation which gives constant values of the velocity constant for the ascending portion of the curve in the following reaction



This reaction had been previously studied by Robert and Soper (4) and Dhar (1) but the order of the reaction has not been determined. Indeed Robert and Soper have pointed out that the reaction is instantaneous as in most ionic reactions and not amenable to kinetical treatment. We find however, that it proceeds regularly if we take into consideration the catalytic activity of colloidal silver (details to be published later).

Now it was Ostwald (see A Text Book of Physical Chemistry by Glasstone) who for the first time studied autocatalysis on a kinetical basis. In the hydrolysis of an ester eg. methyl acetate, the acetic acid produced works as a catalyst. Let  $a$  be the concentration of the ester and  $b$  of the acetic acid present initially and let  $x$  be the amount of ester hydrolysed after time  $t$ , then the rate of hydrolysis at any time is given by

$$\frac{dx}{dt} = k(a-x)(b+x). \quad \dots (1)$$

On integrating in the usual way this gives

$$K = \frac{1}{t(a+b)} \ln \frac{a(b+x)}{b(a-x)} \quad \dots (2)$$

which has been confirmed by Ostwald.

This (2) equation can not be applicable in our case since our reaction is bi-molecular one. We have started with the assumption that for the ascending portion of the curve the colloidal silver produced works as a homogeneous catalyst and is proportional to  $x$ , which is the amount of silver formed. Then

$$\frac{dx}{dt} = k_1(a-x)^2 + k_2(a-x)^2x \quad \dots (3)$$

But since the term  $k_1(a-x)^2$  is negligible as the uncatalysed reaction is very slow we can write

$$\frac{dx}{dt} = k(a-x)^2x \quad \dots (4)$$

Integrating this we get

$$kt = \frac{1}{a^2} \ln \frac{x}{a-x} + \frac{1}{a(a-x)} + c, \quad \dots (5)$$

where  $C$  is the integration constant. It is not possible to find out the value of  $C$ . When we put  $X=0$  when  $t=0$ , we find that the term  $\frac{x}{a-x}$  becomes 0.

The same flaw is observable in Ostwald's equation (2). When  $b$  is zero i.e., when acetic acid present initially is zero then the term  $\frac{a(b+x)}{b(a-x)}$  becomes infinity, and the whole equation becomes meaningless. This can be avoided if we integrate between two time limits when the integration constant disappears. Now

$$\frac{dx}{dt} = k(a-x)^2x$$

and integrating between  $t_2$  and  $t_1$  we get

$$\int_{x_1}^{x_2} \frac{dx}{(a-x)^2x} = k \int_{t_1}^{t_2} dt$$

$$\text{or } \frac{1}{a_2} \int_{x_1}^{x_2} \frac{dx}{x} + \frac{1}{a_2} \int_{x_1}^{x_2} \frac{dx}{(a-x)} + \frac{1}{a} \int_{x_1}^{x_2} \frac{dx}{(a-x)^2} = k \int_{t_1}^{t_2} dt$$

$$\text{or } k(t_2 - t_1) = \frac{1}{a_2} \left\{ \ln \frac{x_2(a-x_1)}{x_1(a-x_2)} \right\} + \frac{1}{a} \left\{ \frac{1}{a-x_2} - \frac{1}{a-x_1} \right\} \quad \dots \dots (6)$$

We find that the equation (6) works well in our case as the following table will show.

$t_2$	$t_1$	$k$
130	50	0.01738
80	30	0.01741
50	30	0.01818
120	80	0.01964
80	50	0.01905

These values are a great improvement over the following values of the constant which have been worked out with usual bimolecular formula viz.,

$K - \frac{1}{t} \frac{x}{a(\bar{a} - x)}$	$k$
$t$	
30	0.001526
50	0.002350
80	0.003287
120	0.008268
180	0.011310

The equation (6) represents a third order reaction although a new type of third order reaction.

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#### References

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8. Krishna and Ghosh, paper read at the joint session of Indian and National Academy of Science, India, Dec. 1946.
4. Robert and Soper, (*J. Chem. Soc.* 2004, 1932).

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